Supplementary figure Legends

Supplementary figure 1. Description of the mouse tumor models. **A.** MC38 tumor model and anti-CTLA4 treatment. **B.** MC38 tumor model and anti-CTLA4/PD-L1 combination therapy. **C.** Cancer vaccination model. **D.** AOM/DSS colon cancer model and anti-CTLA4 treatment. **E.** Fecal transplantation. **F.** Effect of the eradication of *H.p* by antibiotherapy on the efficacy of cancer immunotherapy. **G.** Effect of neutralization of inflammatory cytokines on the efficacy of cancer immunotherapy. **H.** In vivo cytotoxic assay.

Supplementary figure 2. Infectious status of mice. A. Co-housing experiments. At sacrifice, we performed rapid urease tests (RUT) and quantification of *H. pylori* colony forming units (CFU) on the stomach of non-infected (NI) and *H.p* infected (INF) co-housed mice. We only detected *H.p* infection in INF and not in NI mice, confirming that co-housing does not allow for *H.p* transmission. **B.** Antibiotic regimens eradicate *H.p* infection. At sacrifice, we performed RUT and CFU on the stomach of NI, INF and INF mice treated with antibiotics. We did not detect *H.p* infection after antibiotic therapy. **C**. Fecal transplantation. At sacrifice, we performed RUT and CFU on stomach of INF or NI mice transplanted with INF feces. Fecal transplantation does not allow for *H.p* transmission when NI mice were transplanted with feces recovered from INF mice. **D.** MC38 model. At sacrifice, we performed RUT and CFU confirming the infectious status of mice. **F.** Cancer vaccination model. At sacrifice, we performed RUT and CFU confirming the infectious status of mice. AOM/DSS colon cancer model and anti-CTLA4 treatment. At sacrifice, we performed RUT and CFU to confirm the infectious status of mice. Optical densities above the dotted line are considered to be indicative of an active *H.p* infection.

Supplementary figure 3. *H. pylori* infection does not impact $CD8^+$ T cells, $CD4^+$ T cells and DC1 cells at steady state. A. The presence of *H.p* does not affect the absolute cell number and activation states of $CD8^+$ T cells in the blood and spleen of non-infected (NI) and infected (INF)

mice. Absolute number of $CD8^+$ T cells in the blood **(A.1)** and spleen **(A.4).** Activation states of $CD8^+$ T cells; CD44 and CD62L MFIs in the blood **(A.2 & A.3)** and spleen **(A.5 & A.6)**. Similar results were obtained in peripheral lymph nodes (LNs) (data not shown). **B.** The presence of *H.p* does not affect the absolute cell number and activation states of $CD4^+$ T cells or Tregs in the blood and spleen of NI and INF mice. Absolute number of $CD4^+$ T cells in the blood **(B.1)** and spleen **(B.4).** Activation states of $CD4^+$ T cells; CD44 and CD62L MFIs in the blood **(B.2 & B.3)** and spleen **(B.5 & B.6)**. Treg cell number in the blood **(B.7)** and spleen **(B.8)**. Similar results were obtained in peripheral LNs (data not shown). **C.** The presence of *H.p* does not affect the absolute cell number and activation states of migratory DC1 cells in the spleen and peripheral LNs of NI and INF mice. Absolute number of migratory DC1 cells in the spleen **(C.1)** and peripheral LNs **(C.5)**. Activation states; MHCI, CD80 and CD86 MFIs in the spleen **(C.2 – C.4)** and peripheral LNs **(C.6 – C.8)**. **D.** The presence of *H.p* does not affect the absolute cell number and activation states of resident DC1 cells in the spleen and peripheral LNs of NI and INF mice. Absolute number of resident DC1 cells in the spleen **(D.1)** and peripheral LNs **(D.5)**. Activation states; MHCI, CD80 and CD86 MFIs in the spleen **(D.2 – D.4)** and peripheral LNs **(D.6 – D.8)**. Dashed lines represent the MFI of the FMO for each given activation marker. Values below the dashed line are considered as background. Data shown are representative of two independent experiments. No statistical differences were observed between NI and INF mice (Mann-Whitney test). For the experiments described in Supplementary figure 3A-D, the infectious status of each individual mouse was confirmed at sacrifice by performing rapid urease tests (RUT) and/or CFU on the stomach.

Supplementary figure 4. *H. pylori* infection does not impact DC2 cells, moDCs, macrophages and monocytes at steady state. A. The presence of *H.p* does not affect the absolute cell number and activation states of DC2 cells in the spleen and peripheral lymph nodes (LNs) of non-infected (NI) and infected (INF) mice. DC2 cells in the spleen **(A.1 – A.4)** and peripheral LNs **(A.5 – A.8)** of NI and INF mice. Absolute number of DC2 cells in the spleen **(A.1)** and peripheral LNs **(A.5)**.

Activation states of DC2 cells; MHCII, CD80 and CD86 MFIs in the spleen **(A.2 – A.4)** and peripheral LNs **(A.6 – A.8). B.** The presence of *H.p* does not affect the absolute cell number and activation states of monocyte derived DCs (moDCs) in the spleen of NI and INF mice. Absolute number of moDCs (**B.1**). Activation states of moDCs; MHCII, CD80 and CD86 MFIs **(B.2 – B.4)**. **C.** In the spleen, the presence of *H.p* does not affect the absolute cell number and activation states of macrophages in NI and INF mice. Absolute number of macrophages **(C.1)**. Activation states of macrophages; MHCII, CD80 and CD86 MFIs **(C.2 – C.4)**. **D.** In the spleen, the presence of *H.p* does not affect the absolute cell number and activation states of monocytes in NI and INF mice. Absolute number of monocytes **(D.1)**. Activation states of monocytes; MHCII, CD80 and CD86 MFIs **(D.2 – D.4)**. Dashed lines represent the MFI of the FMO for the given activation marker. Values below the dashed line are considered as background. Data shown are representative of two independent experiments. No statistical differences were observed between NI and INF mice (Mann-whitney test). For the experiments described in Supplementary figure 4A-D, the infectious status of each individual mouse was confirmed at sacrifice by performing rapid urease tests (RUT) and/or CFU on the stomach

Supplementary figure 5. Cell counts in each organ/tissue of MC38 tumor-bearing mice 19 days post tumor cell injection. Cell number per mL for blood and per organ for non-draining (ndLN) and tumor draining (tdLN) lymph node, spleen and tumor. No statistical differences were observed between non-infected (NI) and infected (INF) mice (Mann-Whitney test). For the experiments described in Supplementary figure 5, the infectious status of each individual mouse was confirmed at sacrifice by performing rapid urease tests (RUT) and/or CFU on the stomach

Supplementary figure 6. *H. pylori* infection does not affect $CD8^+$ and $CD4^+$ T cells and DC1 cells in MC38 tumor-bearing mice. A. In the tumor draining lymph node (tdLN) and tumor(s) of non-infected (NI) and infected (INF) mice, the presence of *H.p* does not affect the absolute cell

number and activation states of $CD8^+$ T cells. $CD8^+$ T cells in the tdLN **(A.1 – A.3)** and tumor(s) **(A.4 – A.6)** of NI and INF mice. Absolute number of $CD8^+$ T cells in the tdLN **(A.1)** and tumor(s) **(A.4).** Activation states of $CD8^+$ T cells; CD44 and CD62L MFIs in the tdLN **(A.2 & A.3)** and tumor(s) **(A.5 & A.6). B.** In the tdLN and tumor, the presence of *H.p* does not affect the absolute cell number and activation states of $CD4^+$ T cells. $CD4^+$ T cells in the tdLN **(B.1 – B.3)** and tumor **(B.4 – B.6)** of NI and INF mice. Absolute number of $CD4^+$ T cells in the tdLN **(B.1)** and tumor **(B.4).** Activation states of $CD4^+$ T cells; CD44 and CD62L MFIs in the tdLN **(B.2 & B.3)** and tumor **(B.5 & B.6).** Treg cell number in tdLN **(B.7)** and tumor **(B.8)**. **C.** In the tdLN and tumor, the presence of *H.* does not substantially affect the absolute cell number and activation states of migratory DC1 cells. Absolute number of migratory DC1 cells in the tdLN **(C.1)** and tumor **(C.5)**. Activation states; MHCI, CD80 and CD86 MFIs in the tdLN **(C.1 – C.4)** and tumor **(C.6 – C.8)**. **D.** In the tdLN, the presence of *H.p* does not affect the absolute cell number and activation states of resident DC1 cells. Absolute number of resident DC1 cells in the tdLN **(D.1)**. Activation states; MHCI, CD80 and CD86 MFIs in the tdLN **(D.1 – D.4)**. Dashed lines represent the MFI of the FMO for the given activation marker. Values below the dashed line are considered as background. Data shown are representative of two independent experiments. No statistical differences were observed between NI and INF mice (Mann-whitney test). For the experiments described in Supplementary figure 6A-D, the infectious status of each individual mouse was confirmed at sacrifice by performing rapid urease tests (RUT) and/or CFU on the stomach.

Supplementary figure 7. *H. pylori* infection does not impact DC2 cells, moDCs, macrophages and monocytes in MC38 tumor-bearing mice. A. In the tumor draining lymph node (tdLN) and tumor, the presence of *H.p* does not affect the absolute cell number and activation states of DC2 cells. DC2 cell analysis from tdLN **(A.1 – A.4)** and tumor **(A.5 – A.8)** of non-infected (NI) and infected (INF) MC38 tumor-bearing mice. Absolute number **(A.1)** and activation states **(A.2 – A.4)** of DC2 cells in the tdLN. Absolute number **(A.5)** and activation states **(A.6 – A.9)** of DC2 cells in

the tumor. Activation states of DC2 cells; MHCII, CD80 and CD86 MFIs in the tdLN **(A.2 – A.4)** and tumor **(A.6 – A.8). B.** The presence of *H.p* does not affect the absolute cell number and activation states of moDCs and macrophages in the tumor(s) or NI and INF mice. Absolute number of moDCs **(B.1)**. Activation states of moDCs; MHCII, CD80 and CD86 MFIs **(B.2 – B.4)**. Absolute number of macrophages **(B.5)**. Activation states of macrophages; MHCII, CD80 and CD86 MFIs in the spleen **(B.6 – B.8)**. **C.** The presence of *H.p* does not affect the absolute cell number and activation states of monocytes in the tumor(s) of NI and INF mice. Absolute number of monocytes **(C.1)**. Activation states of monocytes; MHCII, CD80 and CD86 MFIs in the tumor **(C.2 – C.4)**. Dashed lines represent the MFI of the FMO for the given activation marker. Values below the dashed line are considered as background. Data shown are representative of two independent experiments. No statistical differences were observed between NI and INF mice (Mann-Whitney test). For the experiments described in Supplementary figure 7A-C, the infectious status of each individual mouse was confirmed at sacrifice by performing rapid urease tests (RUT) and/or CFU on the stomach

Supplementary figure 8. *H. pylori* infection substantially affects tumor myeloid cells in MC38 tumor-bearing mice undergoing anti-CTLA4 immunotherapy. Non-infected (NI) or infected (INF) mice were injected with MC38 colon adenocarcinoma cells (day 0). On days 4, 7 and 11, mice were intraperitoneally injected with anti-CTLA4 treatment or IgG2b isotype as a control. On day 13, mice were sacrificed and the tdLN, ndLN, spleen and tumor(s) were recovered to perform flow cytometric analysis of myeloid and T cell subsets as described in supplementary data FigS3 & 4. The presence of *H.p* does not affect the absolute cell number recovered in tumor(s) of mice undergoing immunotherapy. **A.** Total cell number recovered from tumors. The presence of *H.p* does not affect the absolute cell number of T cells recovered in the tumor(s) of mice undergoing immunotherapy. **B. (B.1 – B.3)** Absolute cell number of $CD3^+$ T cells (total T cell count) **(B.1)**, $CD8^+$ T cells **(B.2)**, and $CD4^+$ T cells **(B.3)**. In the tumor(s) of mice undergoing immunotherapy, the

presence of *H.p* substantially affects the absolute number of myeloid cells ($CD11b^+$), as well as the absolute cell number of activated monocytes. **C.** Absolute cell number of total $CD11b^+$ myeloid cells **(C.1)** and activated monocytes **(C.2)**. Statistical analysis were performed using Mann-Whitney tests. For the experiments described in Supplementary figure 8A-C, the infectious status of each individual mouse was confirmed at sacrifice by performing rapid urease tests (RUT) and/or CFU on the stomach.

Supplementary figure 9. *H. felis* infection does not jeopardize tumor specific immune response.

A. Absolute number and activation status of OT-1 cells isolated from tdLN and tumor of non-infected (NI) and *H. felis* infected mice (*H.f*) of vaccinated B16-OVA tumor-bearing mice (days 10 and 15 post B16-OVA inoculation respectively for LNs and tumors). Activated OT-1 cells were defined has $CD44^+CD62L^-$. **B**. Vaccine-induced cytotoxic activities of OT-1 cells. In vivo cytotoxic activities of OT-1 cells in vaccinated NI and *H.f* infected mice. Statistical analysis had been carried out using the Mann-Whitney test, with $p<0.05$ considered as statistically significant. For the experiments described in supplementary figure 9A and B, the infectious status of each individual mouse was confirmed at sacrifice by performing rapid urease tests (RUT) and/or CFU on the stomach.

Supplementary figure 10. Effect of neutralization of INFγ on the efficacy of vaccine-based cancer immunotherapy. **A.** B16-OVA tumor growth kinetics of non-infected (NI) mice treated with an anti-cancer vaccine (VAC) or PBS as a control. Vaccinated mice were injected with anti-IFNγ neutralizing antibodies or IgG control. Vaccination resulted in decreased tumor growth in mice administered with IgG control, however, did not limit tumor growth in mice injected with anti-IFNγ neutralizing antibodies. At day 15, tumor volumes of vaccinated mice injected with control IgG or anti-IFNγ neutralizing antibodies were statistically different ($p < 0.0001$, two-way ANOVA).

Experimental groups included 6 mice. **B.** Absolute number and activation status of OT-1 cells isolated from tumors of vaccinated mice injected with control IgG or anti-IFNγ neutralizing antibodies (15 post B16-Ova inoculation). Experimental groups included 3 mice. Statistical analysis had been carried out using the Mann-Whitney test, with $p<0.05$ considered as statistically different.

Supplementary figure 11. A. Gating strategy used to define and analyze, by flow cytometry, the absolute number and activation status of $CD8^+$ T cells in the blood, spleen, ndLN, tdLN and tumor of non-infected and infected mice. **(A.1)** Gating on CD45+ lymphocyte population. **(A.2)** Gating on $CD3^+CD8^+$ T cell population. **(A.3)** Activation markers; activated T cells are $CD44^+CD62L^-$ whereas naive T are $CD44^-CD62L^+$. **B.** Gating strategy used to define **(B.2)** migratory and **(B.3)** resident DC1 cells in the spleen, ndLN and tdLN of non-infected (NI) and infected (INF) mice. **C.** Gating strategy used to define **(C.3).** Macrophages and **(C.4)** DC2 cells in the spleen, tdLN and ndLN only and **(C.5)** moDCs in the spleen, tdLN and ndLN of NI and INF mice. **(C.6)** MC38 tumor bearing mice; tumor only; tumor infiltrating DC2 cells are described as $CD64^-$ expressing cells. **D.** Gating strategy for the identification of monocytes in the spleen of NI and INF mice. **(D.1)** After gating on live and single cells, $CD11c^-$ cells were gated on, followed by gating on the **(D.2)** CD11b Ly6C expressing cells.

Supplementary Methods

Flow cytometry. Tumors, lymph nodes and spleen were dissected immediately after sacrifice of tumor-bearing mice. Tumor samples were dissociated manually prior to enzymatic digestion with Liberase TL (Roche) and DNAse (Sigma) in pure RPMI for 45 minutes at 37 °C. Samples were then filtered through a 100-µm cell strainer and washed in RPMI supplemented with 2% FCS.

Lymph nodes and spleen were dissociated manually prior to enzymatic digestion with Liberase TL (Roche) for 15 minutes at 37 °C in pure RPMI for 15 minutes at 37 °C. Samples were then filtered through a 70-µm cell strainer and washed in RPMI supplemented with 2% FCS. Cells were incubated with anti-mouse CD16/32 (clone 24G2) for 10 minutes at room temperature. Cells are then stained for flow cytometry with antibodies to CD45.2-APC-allophycocyanin (APC/Cy7, clone 104, BioLegend, San Diego, CA, USA); CD45.1-Alexafluor700 (AF700, clone A20, BioLegend); CD3-PerCPC5.5 (clone 17A2, BioLegend); CD4-PE-Cy7 (clone GK1.5, BioLegend); CD8-Pacific Blue or CD8-FITC (clone 53-6.7, BioLegend); CD107a-PE (clone 1D4B, BioLegend); CD62L-PE (clone MEL-14, BioLegend); CD44-FITC (clone IM7, BD Pharmingen, San Jose, CA, USA), CD11c-APC/Cy7 (clone N418, BioLegend), CD11b-Pacific Blue (clone M1/70, Biolegend), CD103-PE (clone 2E7, BioLegend), CD80-APC (clone 16-10A1, BioLegend), F4/80-PE-Cy7 (clone BM8, BioLegend), MHCI-A700 (clone AF6-88.5, BioLegend), CD86-PerCPCy5.5 (clone GL-1, BioLegend), MHCII-A700 (clone M5/114.15.2, BioLegend), Foxp3-PE-Cy7 (clone FJK-16s, Invitrogen, Carlsbad, CA, USA), Ly6C-FITC (clone AL-21, BD Pharmingen) or CD64-PE (clone x54-5/7.1, BioLegend). For intracellular staining of Granzyme B, cells were incubated for 6 hours at 37 °C in RPMI supplemented with 10% FCS, 1% Penicillin/Streptomycin, 1 mM sodium pyruvate, 0.05 mM β-mercaptoethanol, 10 mM HEPES in the presence of 10^{-6} M of OVA peptide (SIINFEKL) and Brefeldin A (Biolegend). Cell were then stained with antibodies to CD45.2-APC/Cy7; CD45.1-AF700; CD3-PerCPC5.5; CD4-PE-Cy7; CD8-Pacific Blue; CD107a-PE (clone 1DAB, BioLegend); CD62L-PE; CD44-FITC and Granzyme B (GRZB)-APC (clone QA16AOZ, BioLegend).

For the analysis of dendritic cell activation, tumor-draining lymph nodes of tumor bearing mice were isolated 40 hours after tumor engraftment and processed as described above. Cells were incubated with anti-mouse CD16/32 (clone 24G2) for 10 minutes at room temperature. Cells were then stained with anti-mouse CD11c-APC/Cy7; CD11b-Pacific Blue; CD103-PE and CD80- APC.

For the analysis of the in vivo OT-1 $CD8^+$ T cell proliferation by CFSE dilution, cells were stained with CD45.2-APC/Cy7; CD45.1-AF700; CD3 PE and CD8-Pacific Blue.

Cells were acquired using LRSFortessa cell analyser (BD) or Attune NxT Flow Cytometer (ThermoFisher) and samples were analysed with Flowjo V.07 sorftware (Tree Star Inc., Ashland, OR). See the online supplementary figure S11 for gating strategies used to define and analyze the absolute number and activation status of immune cells.

16S rRNA gene sequence processing and analysis. DNA extraction from feces was performed using ZymoBIOMICS DNA Miniprep Kit (Zymo Research Corporation). Then, DNA was sequenced using 16S ribosomal RNA (rRNA) in order to determine microbiome composition. More precisely, V3 and V4 variable regions were sequenced by P PCR using primers Bakt_341F (5′-CCTACGGGNGGCWGCAG-3′) and Bakt_805R (5′-GACTACHVGGGTATCTAATCC-3′) were adapted to incorporate the transposon-based Illumina Nextera adapters (Illumina) and a sample barcode sequence allowing multiplexed paired-end sequencing. PCR mixtures contained 1× Q5 buffer (NEB), 1× Q5 Enhancer (NEB), 200 µM dNTP (VWR International), 0.2 µM of forward and reverse primer (Integrated DNA Technologies), 1 unit of Q5 (NEB) and 1 µl of template DNA in a 50 µl reaction. The PCR cycling conditions consisted of an initial denaturation step of 30 sec at 98°C, followed by a first set of 15 cycles (98°C for 10 sec, 55°C for 30 sec and 72°C for 30 sec), then by a second step of 15 cycles (98°C for 10 sec, 65°C for 30 sec and 72°C for 30 sec) and final elongation step of 2 min at 72°C before cooling to 4°C indefinitely. PCR products were purified using 35 µl of magnetic beads (AxyPrep Mag PCR Clean up kit; Axygen Biosciences) per 50 µl PCR reaction. Bioanalyzer 2100 DNA 7500 (Agilent Technologies) chips was used for

amplifications control. Subsequently, samples were pooled at an equimolar ratio, then the pool was repurified as and checked for quality on a Bioanalyzer 2100, using a DNA high sensitivity chip. The pool was quantified using picogreen (Life Technologies) and loaded on a MiSeq system (Illumina). High-throughput sequencing was performed at the IBIS (Institut de Biologie Intégrative et des Systèmes - Université Laval). Gene processing followed by analyzation, using R v4.0.0. DADA2R package v1.16.0 (PMID: 27214047) to generate exact amplicon sequence variants (ASV) of each sample from raw amplicon sequences. Sequence errors were determined with the Illumina amplicon sequence errors, de-replicated, chimera removed, and merged of paired-end reads with 260-bases for forward reads and 190-bases for reverse reads. The taxonomy assignment was performed against the SILVA reference database v138 (PMID: 23193283). Generation of graphs and tables was performed at the genus-level through phyloseq R package v1.30.0 (PMID: 23630581). The alpha-diversity was estimated with both the Shannon diversity index and the Inverse Simpson index and compared using Mann-Whitney tests.