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Supplementary Materials for

Repurposing rotavirus vaccines for intratumoral immunotherapy can overcome resistance to immune checkpoint blockade

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Table S4. Genes differentially expressed by A20 tumors in vivo upon active versus inactivated IT rotavirus therapy.

Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/11/515/eaat5025/DC1)

Data file S1 (Microsoft Excel format). Primary data.
Data file S2 (Microsoft Excel format). Gene expression data.

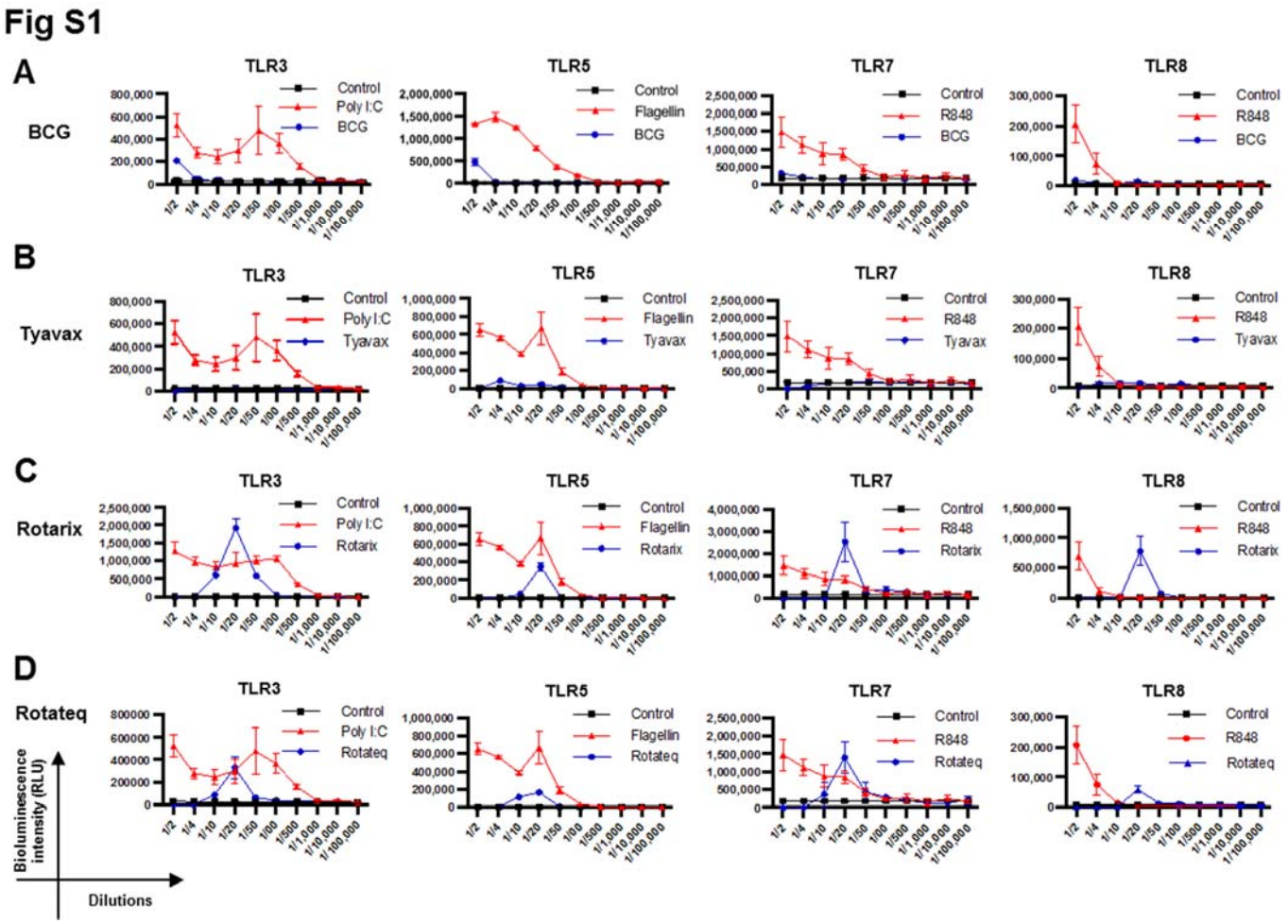


Fig. S1. Proinflammatory features of anti-infectious disease vaccines. NF-κB activation in transgenic 293T cell lines expressing specific Toll-like receptors (TLR-2,3,4,5,7,8) and NF-κB-Luc reporter system by different dilutions of (A) Mycobacterium tuberculosis vaccine (BCG Pasteur), (B) the Typhoid Fever vaccine (Tyavax), (C) & (D) Rotavirus Vaccines (Rotarix and Rotateq respectively). Data are represented as RLU bioluminescence intensity. Red lines represent the activity of the natural ligand for the TLR, blue lines represent the activity of the vaccine and the black line is the negative control (medium). Each data point is mean ± s.e.m; n=3 well per dilution; experiment done with triplicates. The 293T parental cell line does not express any TLR but contains NF-κB reporter system. TLR9 and TLR6 cell lines not depicted because of the lack of positive control activation of the corresponding NF-κB cell lines by the TLR9 and TLR6 synthetic ligands.

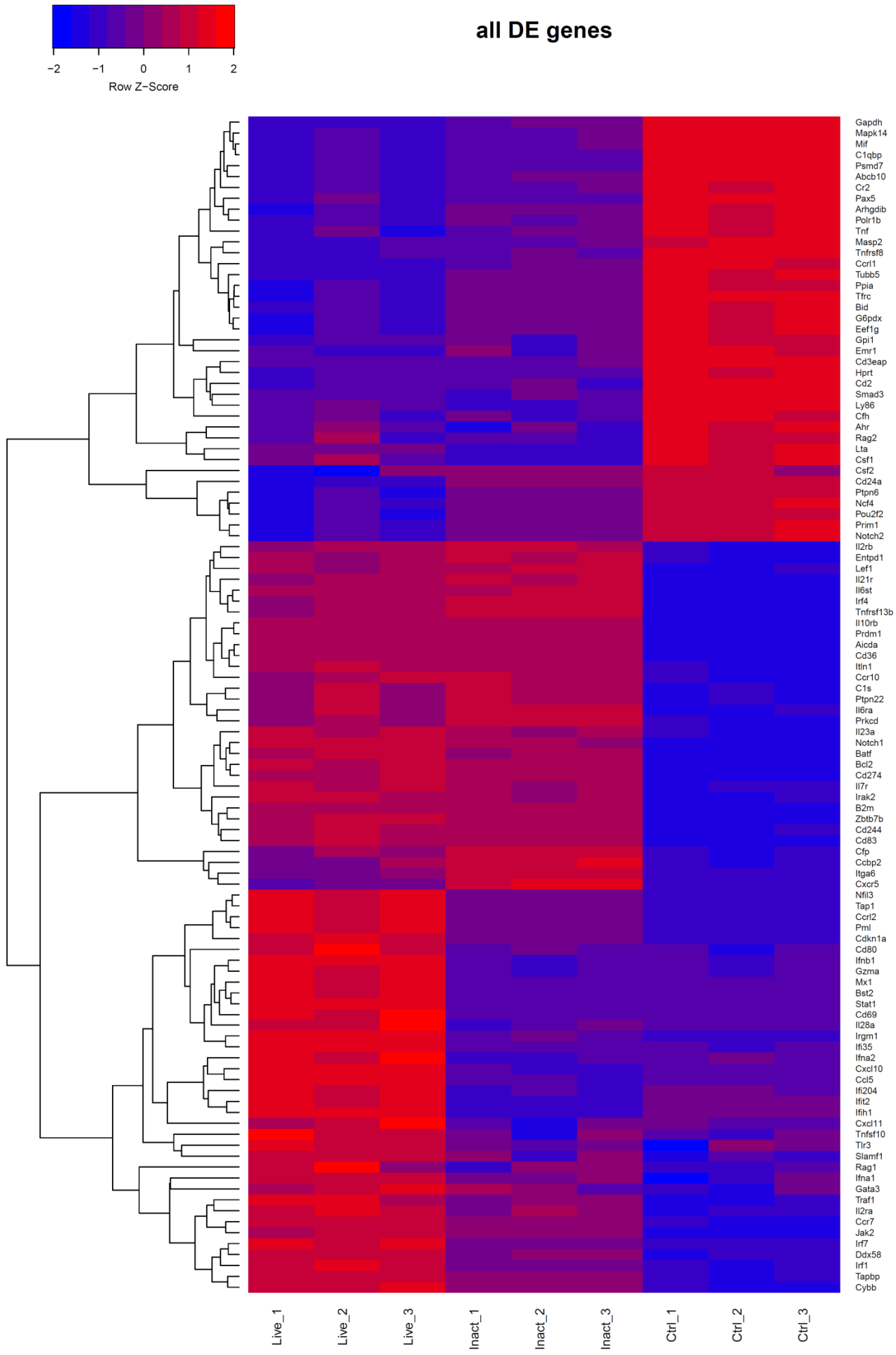

all DE genes
−2
−1
0
1
2
Row Z−Score
Gapdh
Mapk14
Mif
C1qbp
Psmd7
Abcb10
Cr2
Pax5
Arhgdib
Polr1b
Tnf
Masp2
Tnfrsf8
Ccrl1
Tubb5
Ppia
Tfrc
Bid
G6pdx
Eef1g
Gpi1
Emr1
Cd3eap
Hprt
Cd2
Smad3
Ly86
Cfh
Ahr
Rag2
Lta
Csf1
Csf2
Cd24a
Ptpn6
Ncf4
Pou2f2
Prim1
Notch2
Il2rb
Entpd1
Lef1
Il21r
Il6st
Irf4
Tnfrsf13b
Il10rb
Prdm1
Aicda
Cd36
Itln1
Ccr10
C1s
Ptpn22
Il6ra
Prkcd
Il23a
Notch1
Batf
Bcl2
Cd274
Il7r
Irak2
B2m
Zbtb7b
Cd244
Cd83
Cfp
Ccbp2
Itga6
Cxcr5
Nfil3
Tap1
Ccrl2
Pml
Cdkn1a
Cd80
Ifnb1
Gzma
Mx1
Bst2
Stat1
Cd69
Il28a
Irgm1
Ifi35
Ifna2
Cxcl10
Ccl5
Ifi204
Ifit2
Ifih1
Cxcl11
Tnfsf10
Tlr3
Slamf1
Rag1
Ifna1
Gata3
Traf1
Il2ra
Ccr7
Jak2
Irf7
Ddx58
Irf1
Tapbp
Cybb
Live_1
Live_2
Live_3
Inact_1
Inact_2
Inact_3
Ctrl_1
Ctrl_2
Ctrl_3

Fig. S2. Immune gene expression of A20 tumor cells upon in vitro active and inactivated rotavirus exposure. Heatmap displaying the list of all the genes differentially expressed (i.e |log2 fold change|≥2 and p≤0.005) by A20 cancer cells upon 24h exposure with active ("live") or inactivated ("inact") rotavirus in comparison with control (ctrl) media. Experiments done in triplicates; every column displaying the data for each triplicate.

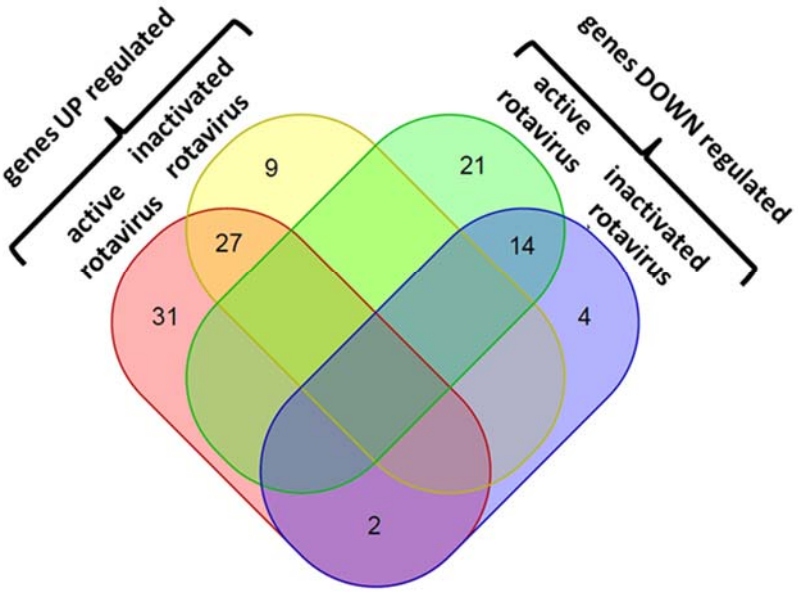


Fig. S3. Number of genes differentially expressed upon exposure of A20 cells with active or inactivated rotavirus in vitro. Venn diagram displaying the number of genes differentially expressed (either up or down regulated) by A20 cancer cells upon 24h exposure with either active or inactivated rotavirus. Mean values of triplicates used for gene selection.

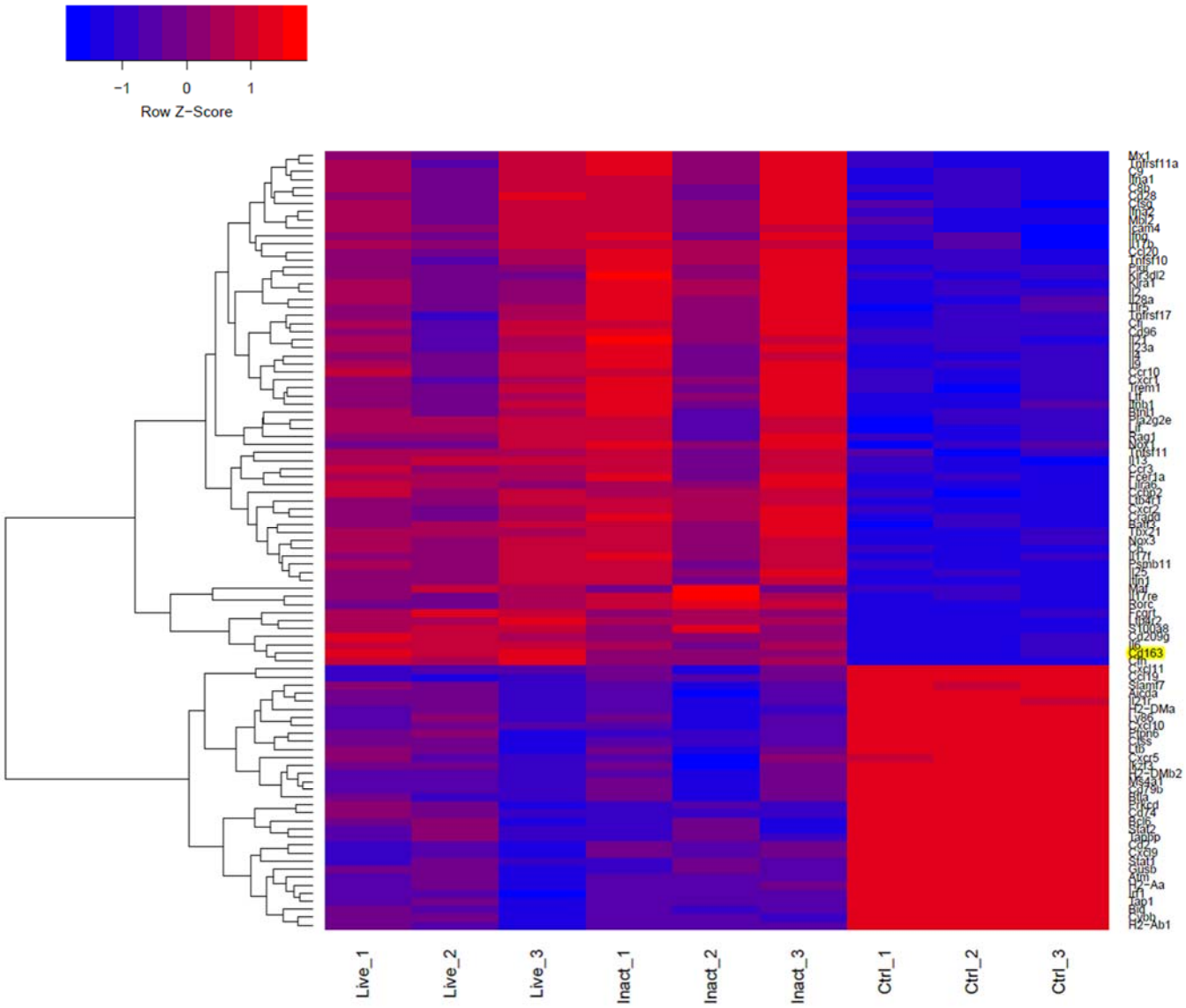
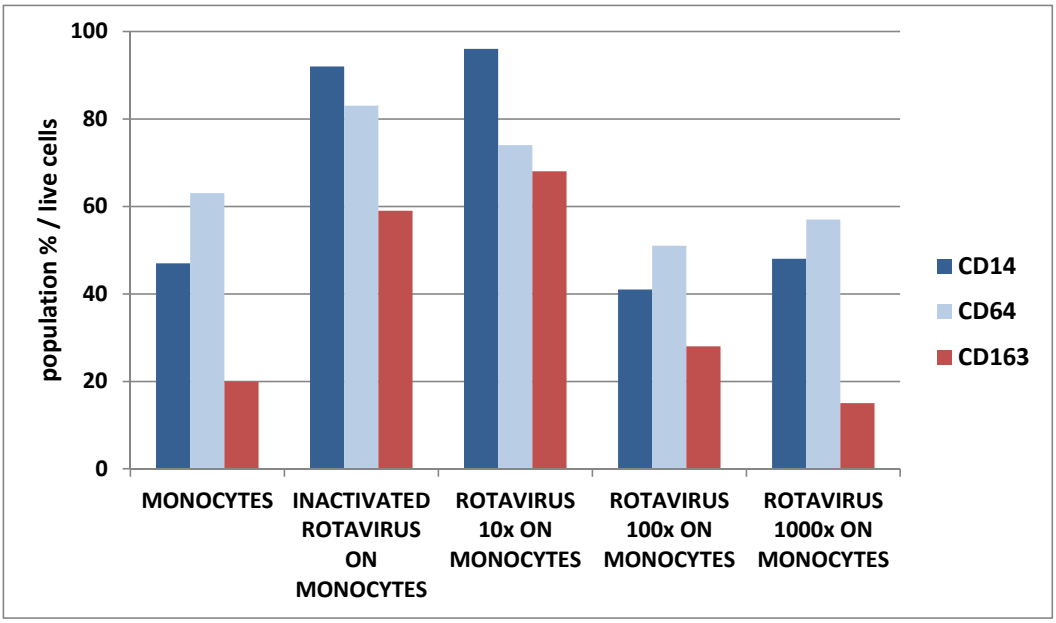
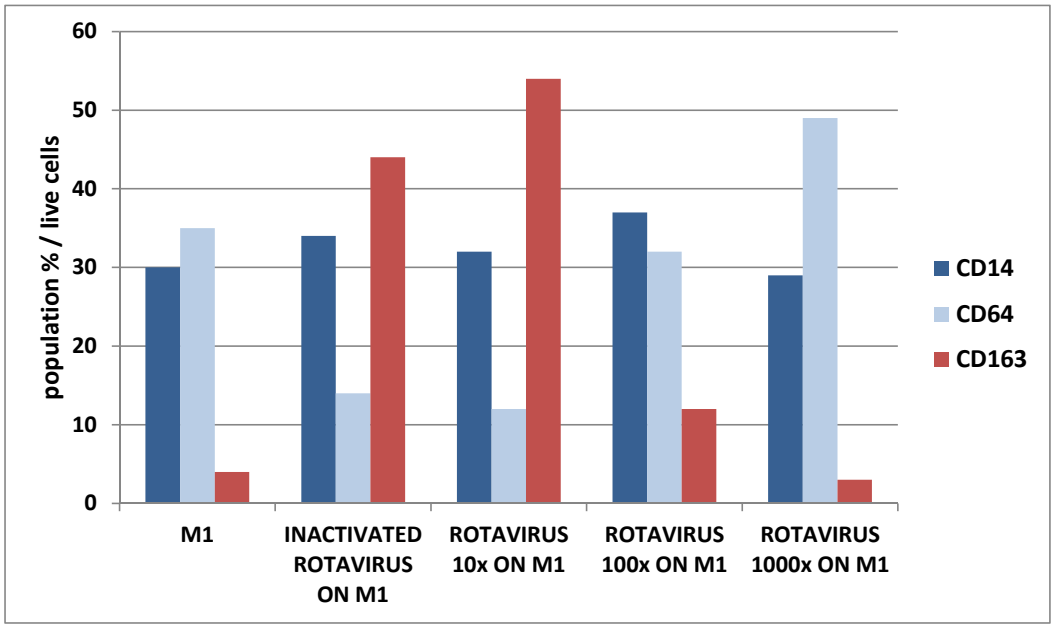


Fig. S4. Immune gene expression of A20 tumors upon in vivo active and inactivated rotavirus exposure. Heatmap displaying the list of the genes differentially expressed (i.e |log2 fold change|≥2 and p≤0.005) by A20 tumors in vivo 48h after IT Rotavirus therapy and commonly regulated by active ("live") or inactivated ("inact") rotavirus in comparison with control (ctrl) media. Experiments done in triplicates; every column displaying the data for each triplicate.


100
80
60
40
20
0
population % / live cells
MONOCYTES
INACTIVATED ROTAVIRUS ON MONOCYTES
ROTAVIRUS 10x ON MONOCYTES
ROTAVIRUS 100x ON MONOCYTES
ROTAVIRUS 1000x ON MONOCYTES
CD14
CD64
CD163


60
50
40
30
20
10
0
population % / live cells
M1
INACTIVATED ROTAVIRUS ON M1
ROTAVIRUS 10x ON M1
ROTAVIRUS 100x ON M1
ROTAVIRUS 1000x ON M1
CD14
CD64
CD163

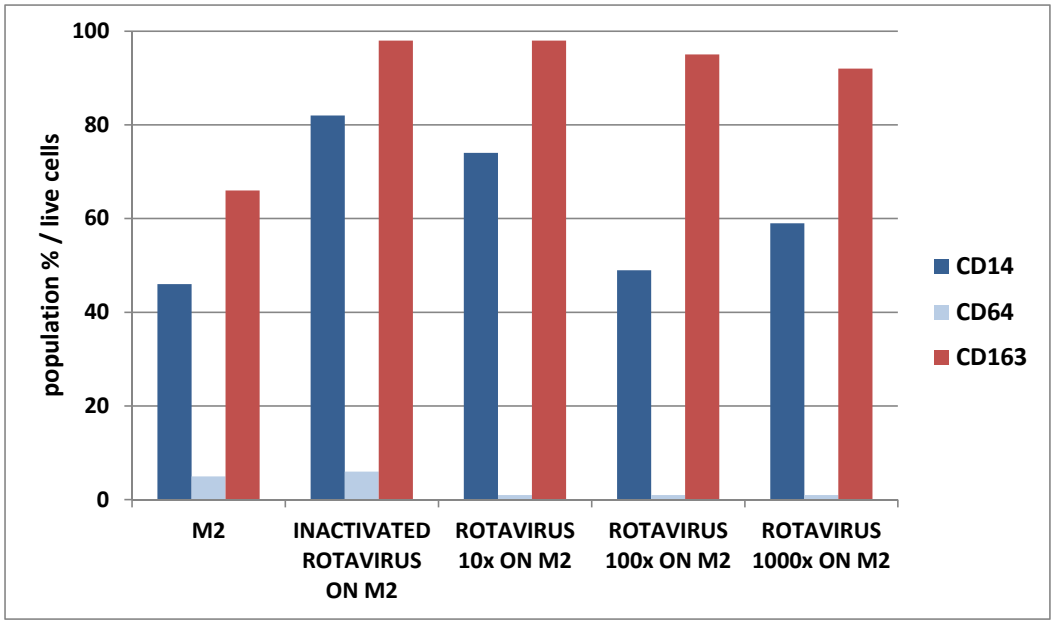

100
80
60
40
20
0
population % / live cells
M2
INACTIVATED ROTAVIRUS ON M2
ROTAVIRUS 10x ON M2
ROTAVIRUS 100x ON M2
ROTAVIRUS 1000x ON M2
CD14
CD64
CD163

Fig. S5. Impact of in vitro rotavirus exposure on human myeloid cells. Flow cytometry analysis of the proportion (among live cells) of CD14, CD64, and CD163 expression on human monocytes and PBMC-derived macrophages (M1 and M2), incubated inactivated Rotavirus or growing concentrations of active rotavirus for 48h.For every condition, a minimal number of 10,000 events have been collected by flow cytometry.

Table S1. Contingency table of the number of genes differentially expressed (steady, up-regulated, or down-regulated) between EMT6 and A20 cancer cell lines upon rotavirus exposure. Experiments run in vitro in triplicates (3 wells with rotavirus for 24h vs 3 wells with media for each cancer cell line).

		EMT6		
		steady	down	up
A20	steady	0	25	40
	down	31	2	2
	up	34	3	23

Table S2. Number of genes commonly regulated between A20 and EMT6 cancer cell lines upon rotavirus exposure.

	Number of Genes Differentially expressed		Number of genes in common	p value (Two sided Fisher test)
	A20 vs CTRL	EMT6 vs CTRL		
UP + DOWN	65	65	30	6.32 E-04
UP only	37	42	23	2.15 E-08
DOWN only	33	28	2	7.05 E-01

Table S3. Number of genes commonly regulated upon exposure of A20 cancer cells with active rotavirus or inactivated rotavirus in comparison with control media.

A20 Cancer Cells In Vitro	Number of Genes		Number of genes commonly regulated	p value (Two sided Fisher test)
	Active Rotavirus vs CTRL	inactive Rotavirus vs CTRL		
UP + DOWN	52	13	43	7.54 E-24
UP only	33	9	27	4.33 E-21
DOWN only	21	6	14	3.45 E-14

gene	live.lfc	inact.lfc	live.pval	inact.pval
Cd163	3.43	1.85	3.40E-71	5.40E-19
Nox3	2.4	2.7	2.30E-13	1.10E-09
Cd209g	2.23	1.49	4.80E-24	8.90E-14
Ifna1	2.09	2.67	1.10E-14	1.50E-11
Il25	2	2.4	2.00E-12	2.50E-09
Il6	2	1.37	5.70E-21	7.20E-06
Ltb4r1	1.97	2.49	7.20E-12	7.10E-20
C9	1.96	2.43	1.60E-08	2.20E-08
S100a8	1.92	1.82	5.60E-28	6.80E-09
Psmb11	1.9	1.99	2.20E-10	1.60E-06
Mbl2	1.88	2.35	1.90E-08	1.20E-07
Il9	1.87	2.28	8.10E-09	1.30E-06
Cxcr2	1.79	2.55	3.70E-08	5.40E-12
Cfh	1.77	1.28	1.80E-18	4.80E-10
Icam4	1.77	1.95	4.10E-08	1.70E-07
Fcgrt	1.75	1.17	1.60E-19	3.20E-07
Ifna2	1.75	2.23	2.60E-07	5.00E-08
Mx1	1.71	2.54	1.60E-05	4.70E-11
Ltb4r2	1.7	1.55	5.20E-13	1.30E-10
Maf	1.69	1.93	2.20E-15	1.90E-04
Itln1	1.68	1.87	7.40E-14	7.60E-08
Tnfrsf11a	1.67	2.4	6.20E-10	3.80E-10
Il17f	1.66	2.08	2.40E-09	2.50E-08
Klra1	1.66	2.73	4.10E-05	2.90E-10
Tbx21	1.66	1.92	2.30E-10	4.90E-07
Pla2g2e	1.65	1.6	5.70E-09	2.70E-04
Cfi	1.62	2.38	5.50E-06	5.00E-09
C8b	1.6	1.89	3.00E-08	1.00E-05
Fcer1a	1.57	1.85	2.00E-07	5.50E-06
Il17b	1.55	1.9	2.30E-06	1.10E-07
Ltf	1.54	2.17	1.40E-07	2.30E-07
Il2	1.49	2.43	1.20E-05	2.90E-10
C6	1.47	1.61	6.80E-10	2.30E-07
Il17re	1.45	2.83	3.20E-06	1.30E-12
Trem1	1.45	1.96	6.90E-06	4.40E-06
Il4	1.37	1.7	4.50E-06	3.10E-05
Ifnb1	1.35	1.97	1.90E-05	6.70E-06
Ctsg	1.33	1.98	3.60E-04	5.40E-06
Nox1	1.32	1.89	7.10E-05	4.20E-06
Il28a	1.25	2.11	7.50E-06	6.40E-07
Cxcr1	1.21	2.21	2.30E-04	1.40E-07
Il23a	1.19	1.86	1.50E-04	2.30E-05
Tnfrsf17	1.19	2.14	4.20E-04	3.60E-08
Cd96	1.17	1.85	1.10E-04	2.20E-06
Il21	1.14	1.77	1.40E-04	5.20E-06
Pigr	1.14	2.18	1.20E-04	1.50E-07
Ifng	1.11	1.58	6.10E-04	2.80E-04
Rorc	1.09	1.91	1.00E-05	2.90E-15
Tlr5	1.09	1.84	2.60E-04	2.00E-06
Tnfsf10	1.08	1.92	2.70E-04	9.20E-08
Kir3dl2	1.06	2.43	7.60E-04	3.10E-08
Ccl20	1.04	1.9	4.40E-04	7.80E-08
Cradd	1.03	1.63	4.80E-05	1.90E-08
Slamf7	-1.14	-1.57	7.10E-08	5.80E-09
Prkcd	-1.15	-1.55	5.70E-08	1.40E-20
Stat2	-1.28	-1.65	4.70E-09	3.90E-09
Cd74	-1.3	-1.69	2.00E-10	5.30E-28
Ctss	-1.45	-1.56	9.90E-11	5.40E-11
Bid	-1.53	-1.42	3.10E-15	2.90E-12
Il21r	-1.54	-1.93	1.60E-15	1.50E-09
Tap1	-1.57	-1.41	5.40E-16	1.10E-18
Cxcl11	-1.64	-1.2	1.60E-15	6.20E-05
Cybb	-1.64	-1.85	6.10E-15	1.00E-22
Aicda	-1.65	-1.99	4.60E-15	1.30E-07
Ikzf3	-1.71	-1.56	1.10E-17	4.60E-05
H2-DMb2	-1.73	-1.57	5.20E-20	1.50E-07
H2-Aa	-1.81	-1.62	1.30E-15	2.30E-18
Irf1	-2.04	-1.7	3.60E-19	5.80E-26
Ms4a1	-2.08	-1.92	4.10E-30	9.30E-08
Cd79b	-2.31	-2.02	5.70E-35	4.80E-08
Cxcl9	-2.76	-2	1.10E-49	3.50E-21
Ccl19	-3.07	-1.87	1.20E-58	9.80E-17

Table S4. Genes differentially expressed by A20 tumors in vivo upon active versus inactivated IT rotavirus therapy. List (and heatmap ranking) of genes differentially expressed (i.e |log2 fold change|≥2 or "lfc" and p≤0.005) by A20 tumors in vivo 48h after IT Rotavirus therapy and commonly regulated by active ("live") or inactivated ("inact") rotavirus in comparison with control (ctrl) media. Mean values of triplicates displayed in fig. S4.