Additional File 1
(Associated with Fig. 1)

Additional File 1. Mouse IDO1-derived peptides

Peptide	Sequence	MHC Restriction	SYFPEITHI Score	RANKPEP % Optimal
EP1	MPPAHRNFL	$H2L^d$	23	27.38
EP2	LPTLSTDGL	$H2L^d$	23	23.28
EP3	DPDTFFHVL	$H2L^d$	23	28.45
EP4	IFQSLDVLL	$H2K^d$	23	22.93
EP5	AYNECVNGL	$H2K^d$	21	45.93
EP6	VSLLVEIAASPAIKA	IA^d	30	ND
EP7	VDTYIMKPSKKKPTD	IE^d	32	ND

Additional File 2
(Associated with Fig. 1)

```
MuIDO1      1                  MALSKISPTEGSRRILEDHHIDEDVGFALPHPLVELPDAYSPWVLVA      47
HuIDO1      1                      MAHAMENSWTISKEYHIDEEVGFALPNPQENLPDFYNDWMFIA      43

                                  EP2
MuIDO1     48    RNLPVLIENGQLREEVEKLPTLSTDGLRGHRLQRLAHLALGYITMAYVWNRGDDDVRKVL      107
HuIDO1     44    KHLPDLIESGQLRERVEKLNMLSIDHLTDHKSQRLARLVLGCITMAYVWGKGHGDVRKVL      103

MuIDO1     108   PRNIAVPYCELSEKLGLPPILSYADCVLANWKKKDPNGPMTYENMDILFSFPGGDCDKGF      167
HuIDO1     104   PRNIAVPYCQLSKKLELPPILVYADCVLANWKKKDPNKPLTYENMDVLFSFRDGDCSKGF      163

                EP6                                                      EP3
MuIDO1     168   FLVSLLVEIAASPAIKAIPTVSSAVERQDLKALEKALHDIATSLEKAKEIFKRMRDFVDP      227
HuIDO1     164   FLVSLLVEIAAASAIKVIPTVFKAMQMQERDTLLKALLEIASCLEKALQVFHQIHDHVNP      223

                                                        EP4
MuIDO1     228   DTFFHVLRIYLSGWKCSSKLPEGLLYEGVWDTPKMFSGGSAGQSSIFQSLDVLLGIKHEAGK    289
HuIDO1     224   KAFFSVLRIYLSGWKGNPQLSDGLVYEGFWEDPKEFAGGSAGQSSVFQCFDVLLGIQQTAGG    285

                          EP1                                   EP5
MuIDO1     290   ESPAEFLQEMREYMPPAHRNFLFFLESAPPVREFVISRHNEDLTKAYNECVNGLVSVRKFH      350
HuIDO1     286   GHAAQFLQDMRRYMPPAHRNFLCSLESNPSVREFVLSKGDAGLREAYDACVKALVSLRSYH      346

                EP7
MuIDO1     351   LAIVDTYIMKPSKKKPTDGDKSEEPSNVESRGTGGTNPMTFLRSVKDTTEKALLSWP         407
HuIDO1     347   LQIVTKYILIPASQQPKENKTSEDPSKLEAKGTGGTDLMNFLKTVRSTTEKSLLKEG         403
```

Additional File 2. Location of experimental peptides within the murine IDO1 amino acid sequence and alignment with human IDO1. Peptide identifiers are included in bold at the top and beginning of each sequence. Predicted MHC class I-directed peptide sequences (9-mers) are highlighted in yellow, predicted MHC class II-directed sequences (15-mers) are highlighted in green. IDO1 protein sequences are from Metz et al. 2007 Cancer Res. 67:7082.

Additional File 3 (1 of 4)
(Associated with Fig. 1)

A

EP1

Sample Name : MHC class I mIDO1 1
Sample ID : U0153BC180-5

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow:1 ml/min
Wavelength:220 nm

Time	Unit	Command	Value	Comment
0.01	Pumps	Pump A B.Conc	5	
25.00	Pumps	Pump A B.Conc	65	
25.01	Pumps	Pump A B.Conc	95	
31.00	Pumps	Pump A B.Conc	95	
31.01	Pumps	Pump A B.Conc	5	
40.00	Pumps	Pump A B.Conc	5	
40.01	Controller	Stop		

<<Column Performance>>
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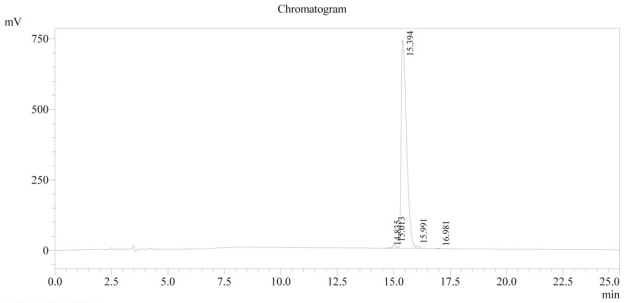
Column : AlltimaTM C18 4.6 x 250 mm

Chromatogram



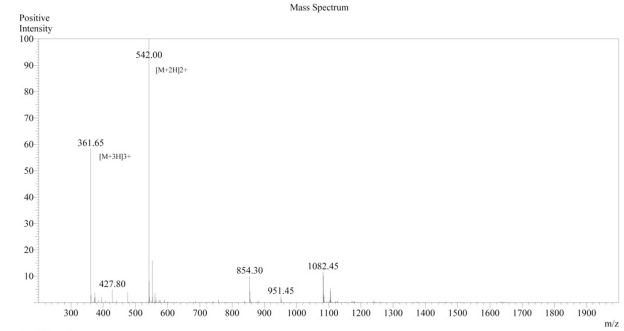
1 Det.A Ch1 / 220nm

Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	14.835	15725	1723	0.127
2	15.013	120236	14556	0.970
3	15.394	12154622	736696	98.105
4	15.991	90697	8339	0.732
5	16.981	8070	1169	0.065
Total		12389349	762483	100.000

Mass Spectrum



Sample Information
Acquired by : Gary
Month-Day Processed : 04/01/16
Time Processed : 13:54:27
Injection Volume : 0.2
Sample Name : MHC class I mIDO1 1
Sample ID : U0153BC180-5
Theoretical MW : 1082.28
Observed MW : 1082.00

Interface :ESI
Nebulizing Gas Flow :1.5L/min
CDL Temp :250℃
Block Temp :200℃

Interface Bias : +4.5 kV
Drying Gas Flow :5 L/min
T.Flow :0.2 ml/min
B.conc :50%H2O/50%MeOH

B

EP2

Sample Name :MHC class I mIDO1 2
Sample ID :U1689DK150-1
Time Processed : 7:55:18
Month-Day-Year Processed : 11/23/2018

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow:1 ml/min
Wavelength:220 nm

Time	Unit	Command	Value	Comment
0.01	Pumps	Pump B Conc.	5	
25.00	Pumps	Pump B Conc.	65	
25.01	Pumps	Pump B Conc.	95	
27.00	Pumps	Pump B Conc.	95	
27.01	Pumps	Pump B Conc.	5	
35.00	Pumps	Pump B Conc.	5	
35.01	Controller	Stop		

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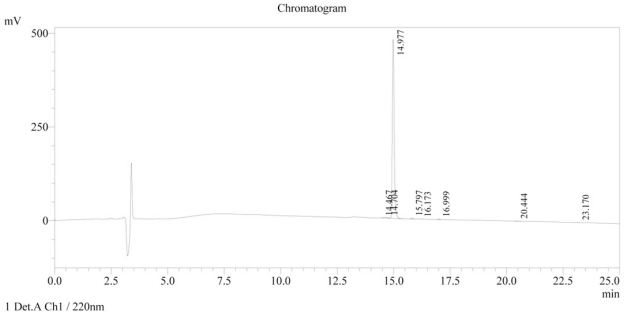
Column : Inertsil ODS-3 4.6 x 250 mm
Equipment: G09010878

Chromatogram



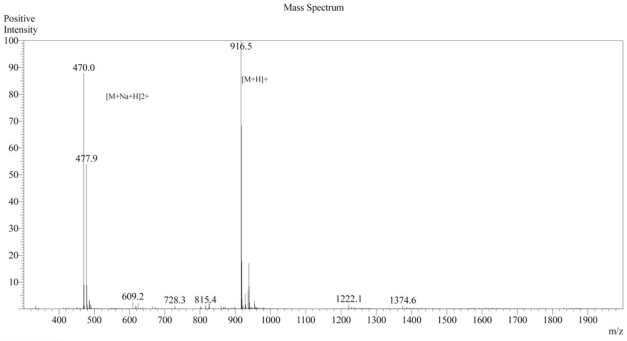
1 Det.A Ch1 / 220nm

Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	14.467	265	76	0.010
2	14.704	20076	1781	0.724
3	14.977	2724798	477342	98.332
4	15.797	13076	2381	0.472
5	16.173	1118	192	0.040
6	16.999	7285	1278	0.263
7	20.444	3273	357	0.118
8	23.170	1123	120	0.041
Total		2771014	483527	100.000

Mass Spectrum



Sample Information
Acquired by : Gary
Month-Day Processed : 11/22/18
Time Processed : 05:24:25 PM
Injection Volume : 0.1
Sample Name : MHC class I mIDO1 2
Sample ID : U1689DK150-1
Theoretical MW : 916.03
Observed MW : 915.5

Interface :ESI
Nebulizing Gas Flow :1.5L/min
CDL Temp :250
Block Temp :200

Equipment : GK11010007
Interface Bias : +4.5 kV
Drying Gas Flow :5 L/min
T.Flow :0.2 ml/min
B.conc :50%H2O/50%MeOH

Additional File 3 (2 of 4)
(Associated with Fig. 1)

C

EP3

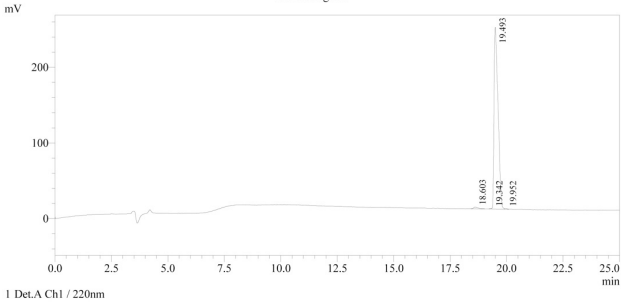
Sample Name : MHC class I mIDO1 3
Sample ID : U0153BC180-9

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow:1 ml/min
Wavelength:220 nm

Time	Unit	Command	Value	Comment
0.01	Pumps	Pump A B.Conc	5	
25.00	Pumps	Pump A B.Conc	65	
25.01	Pumps	Pump A B.Conc	95	
31.00	Pumps	Pump A B.Conc	95	
31.01	Pumps	Pump A B.Conc	5	
40.00	Pumps	Pump A B.Conc	5	
40.01	Controller	Stop		

<<Column Performance>>
<Detector A>
Column : AlltimaTM C18 4.6 x 250 mm

Chromatogram



1 Det.A Ch1 / 220nm

Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	18.603	34729	2395	1.159
2	19.342	4686	883	0.156
3	19.493	2949997	240342	98.421
4	19.952	7924	1049	0.264
Total		2997335	244669	100.000

D

EP4

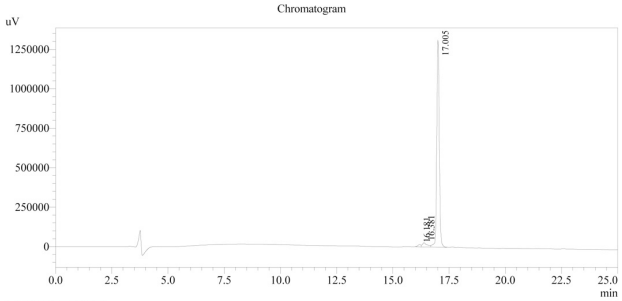
Sample Name :MHC class I mIDO1 4
Sample ID :U0153BC180-11

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow:1 ml/min
Wavelength:220 nm

Time	Unit	Command	Value	Comment
0.01	Pumps	Pump B Conc.	5	
25.00	Pumps	Pump B Conc.	65	
25.01	Pumps	Pump B Conc.	95	
31.00	Pumps	Pump B Conc.	95	
31.01	Pumps	Pump B Conc.	5	
40.00	Pumps	Pump B Conc.	5	
40.01	Controller	Stop		

<<Column Performance>>
<Detector A>
Column : AlltimaTM C18 4.6 x 250 mm

Chromatogram



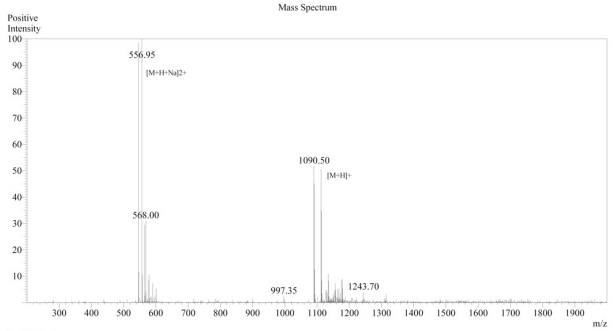
1 Det.A Ch1 / 220nm

Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	16.181	128521	13695	1.200
2	16.381	398475	26583	3.722
3	17.005	10179690	1312608	95.078
Total		10706686	1352885	100.000

Mass Spectrum

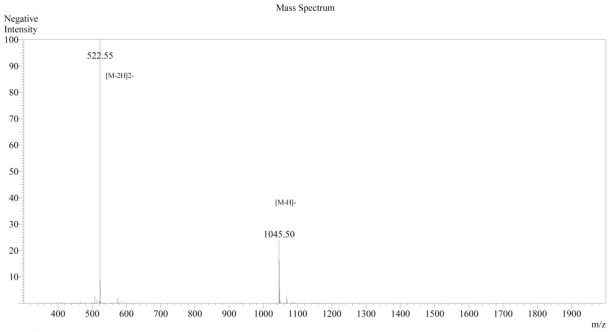


Sample Information
Acquired by : Gary
Month-Day Processed : 03/26/16
Time Processed : 15:46:56
Injection Volume : 0.2
Sample Name : MHC class I mIDO1 3
Sample ID : U0153BC180-9
Theoretical MW : 1090.19
Observed MW : 1090.90

Interface :ESI
Nebulizing Gas Flow :1.5L/min
CDL Temp :250℃
Block Temp :200℃

Interface Bias : +4.5 kV
Drying Gas Flow :5 L/min
T.Flow :0.2 ml/min
B.conc :50%H2O/50%MeOH

Mass Spectrum



Sample Information
User : Gary
Month-Day Processed : 03/28/16
Time Processed : 10:42:27
Injection Volume : 0.2
Sample Name : MHC class I mIDO1 4
Sample ID : U0153BC180-11
Theoretical MW : 1047.25
Observed MW : 1047.10

Interface :ESI
Nebulizing Gas Flow :1.5L/min
CDL Temp :250℃
Block Temp :200℃

Interface Bias : -3.5 kV
Drying Gas Flow :5 L/min
T.Flow :0.2 ml/min
B.conc :50%H2O/50%MeOH

Additional File 3 (3 of 4)
(Associated with Fig. 1)

E

EP5

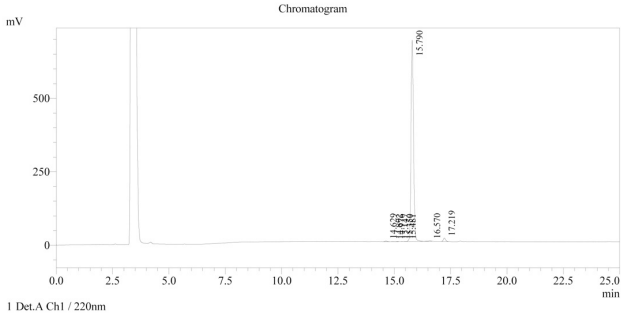
Sample Name : MHC class I mIDO1 5
Sample ID : U0153BC180-13

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow:1 ml/min
Wavelength:220 nm

Time	Unit	Command	Value	Comment
0.01	Pumps	Pump A B.Conc	5	
25.00	Pumps	Pump A B.Conc	65	
25.01	Pumps	Pump A B.Conc	95	
31.00	Pumps	Pump A B.Conc	95	
31.01	Pumps	Pump A B.Conc	5	
40.00	Pumps	Pump A B.Conc	5	
40.01	Controller	Stop		

<<Column Performance>>
<Detector A>
Column : AlltimaTM C18 4.6 x 250 mm

Chromatogram



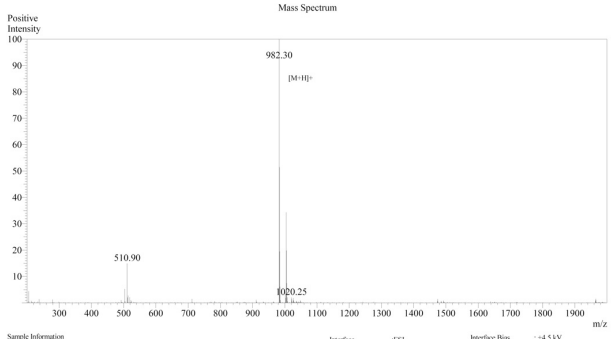
1 Det.A Ch1 / 220nm

Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	14.629	11666	1662	0.218
2	14.892	435	105	0.008
3	14.976	470	127	0.009
4	15.147	2509	338	0.047
5	15.350	1017	193	0.019
6	15.481	1478	298	0.028
7	15.790	5240046	685136	97.935
8	16.570	14958	1448	0.280
9	17.219	77941	11236	1.457
Total		5350520	700541	100.000

Mass Spectrum



Sample Information
Acquired by : Gary
Month-Day Processed : 03/28/16
Time Processed : 11:24:14
Injection Volume : 0.2
Sample Name : MHC class I mIDO1 5
Sample ID : U0153BC180-13
Theoretical MW : 982.07
Observed MW : 981.30

Interface :ESI
Nebulizing Gas Flow :1.5L/min
CDL Temp :250℃
Block Temp :200℃

Interface Bias : +4.5 kV
Drying Gas Flow :5 L/min
T.Flow :0.2 ml/min
B.conc :50%H2O/50%MeOH

Additional File 3 (4 of 4)
(Associated with Fig. 1)

F

Sample Name :MHC class II mIDO1 6
Sample ID :U1689DK150-3
Time Processed :13:43:35
Month-Day-Year Processed :11/25/2018

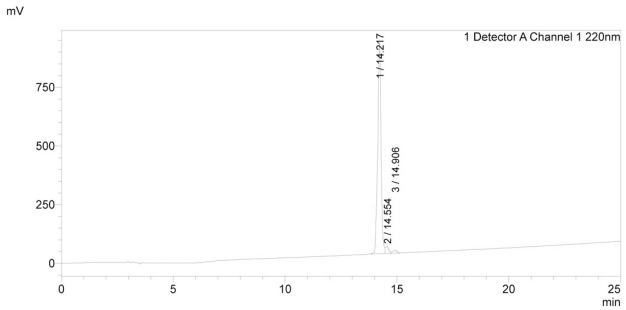
EP6

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow:1 ml/min
Wavelength:220 nm
<<LC Time Program>>

Time	Module	Command	Value
0.01	Pumps	Pump A B.Conc	15
25.00	Pumps	Pump A B.Conc	75
25.01	Pumps	Pump A B.Conc	95
32.00	Pumps	Pump A B.Conc	95
32.01	Pumps	Pump A B.Conc	15
40.00	Pumps	Pump A B.Conc	15
40.00	Controller	Stop	

<<Column Performance>>
<Detector A>
Column : AlltimaTM C18 4.6 x 250 mm
Equipment:ZJ17010508

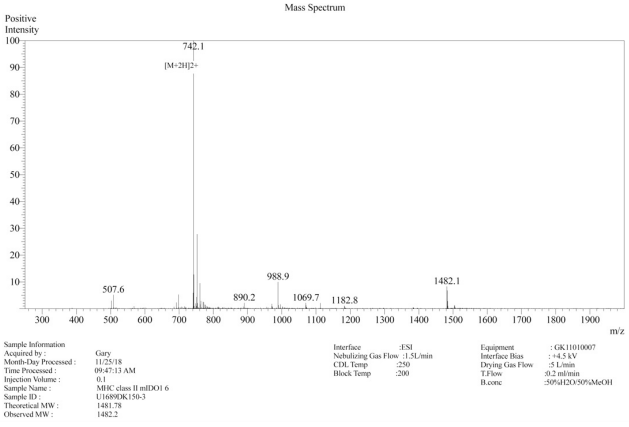
<Chromatogram>



<Peak Table>

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	14.217	9284044	898654	95.336
2	14.554	295838	29072	3.038
3	14.906	158335	12448	1.626
Total		9738217	940174	100.000



G

Sample Name : MHC class II mIDO1 7
Sample ID : U3264BH220-7

EP7

Pump A : 0.065% trifluoroacetic in 100% water (v/v)
Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)
Total Flow:1 ml/min
Wavelength:220 nm

Time	Unit	Command	Value	Comment
0.01	Pumps	Pump A B.Conc	5	
25.00	Pumps	Pump A B.Conc	65	
25.01	Pumps	Pump A B.Conc	95	
31.00	Pumps	Pump A B.Conc	95	
31.01	Pumps	Pump A B.Conc	5	
40.00	Pumps	Pump A B.Conc	5	
40.10	Controller	Stop		

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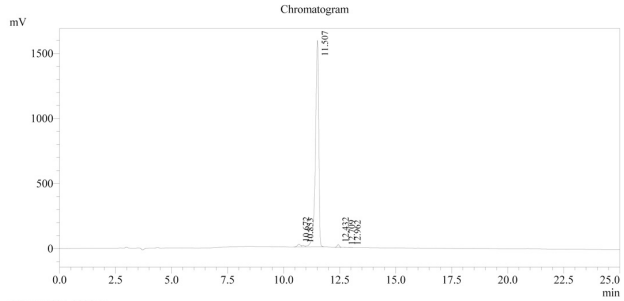
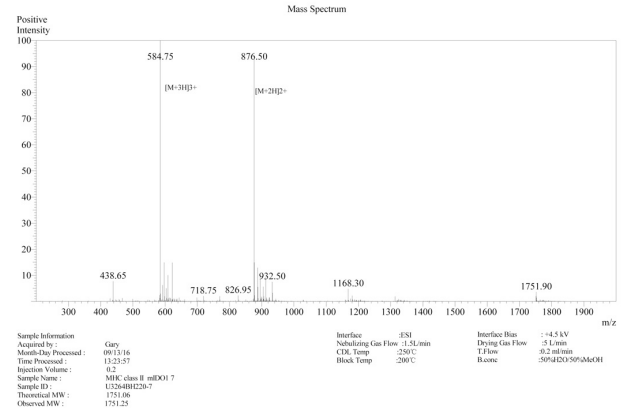
<Detector A>
Column : AlltimaTM C18 4.6 x 250 mm



Peak Table

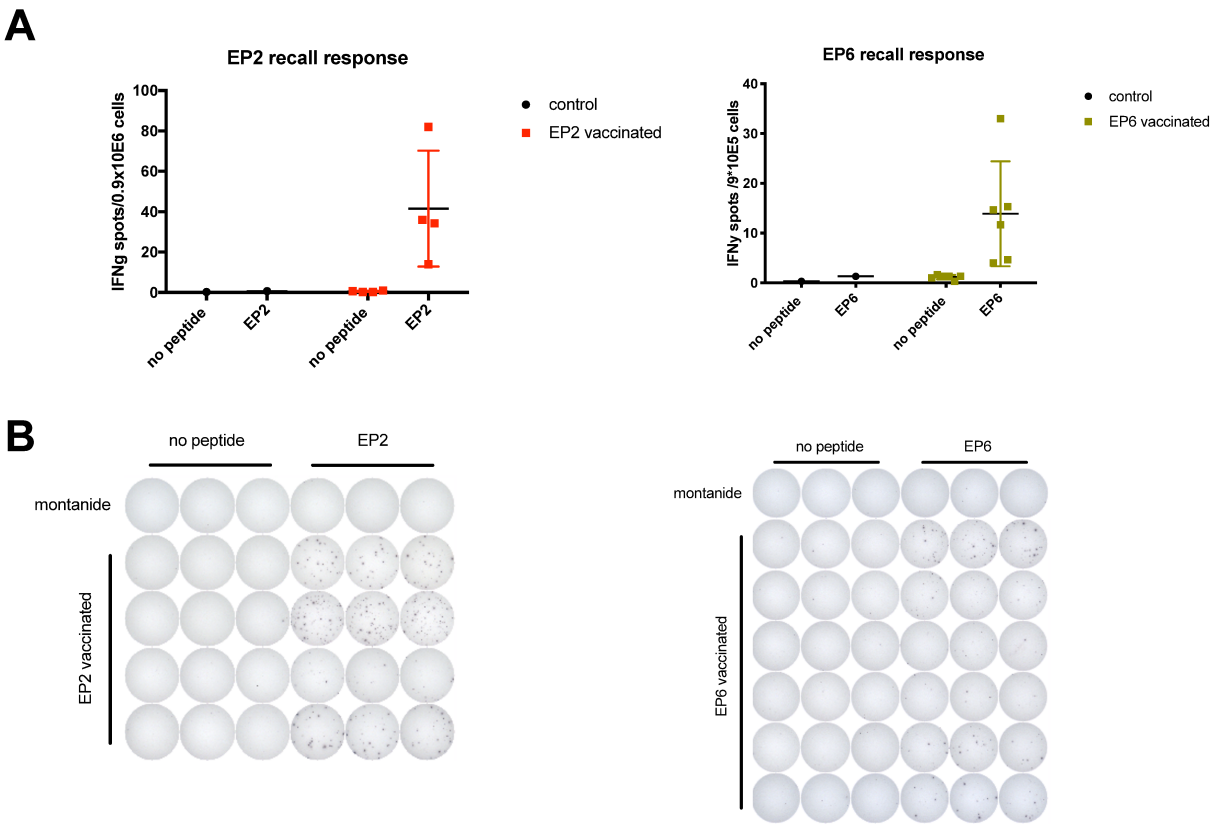
Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	10.672	203698	19751	1.227
2	10.853	95127	12369	0.573
3	11.507	16144872	1586294	97.256
4	12.432	151067	21845	0.910
5	12.709	3442	591	0.021
6	12.962	2229	355	0.013
Total		16600434	1641204	100.000



Additional File 3. Quality control reports for custom synthesized peptides provided by GenScript. (**A-G**) HPLC and MS reports for peptides EP1-7 described in Table 1. (*top*) Reversed-phase high performance liquid chromatography analysis of peptide purity. All peaks shown in the chromatogram are listed according to their retention time. The Area % of the target peptide was used to calculate the peptide HPLC purity. (*bottom*) Electrospray ionization mass spectrometry (ESI-MS) analysis to confirm target peptide molecular weights. In positive ionization mode, the m/z value of the target peptide can be used to calculate the measured molecular weight (MW) by using the formula MW=(m/z)*|z|-z. The MW was calculated from the most abundant m/z peak of the analyte.

Additional File 4
(Associated with Fig. 1)

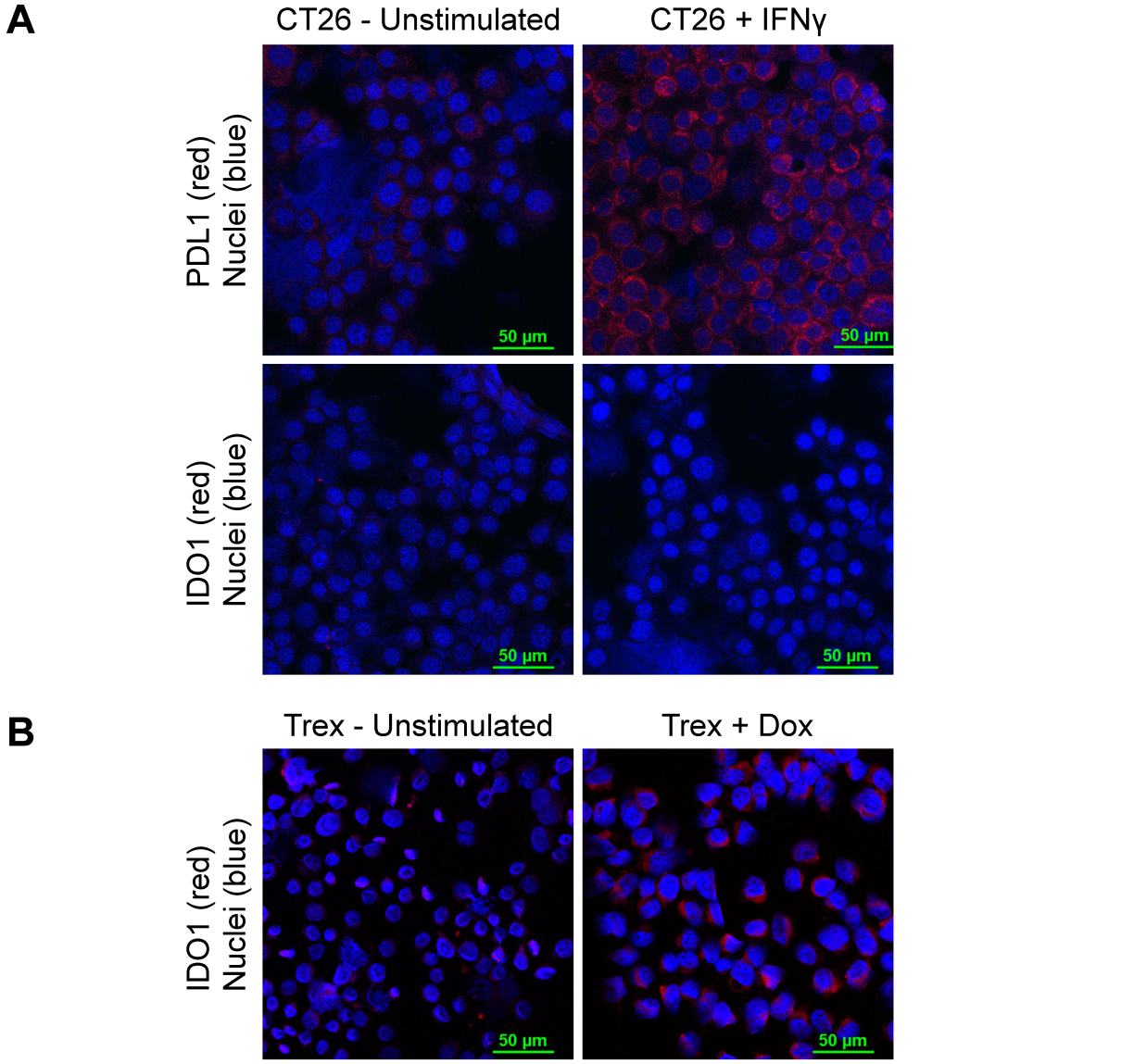


Additional File 4. T cell responses to MHC class I and II-directed, IDO1 peptides measured by IFNγ ELISPOT. (**A**) Quantitative comparisons of IFNγ-producing splenocytes. Cells from mice injected with Montanide ± peptide on d0 and d7 were isolated on d14 and placed in culture ± peptide to assess the degree of peptide-directed recall response, *left* EP2 peptide, *right* EP6 peptide. All datasets are graphed as means ± SD. (**B**) Individual ELISPOT plates from which the data were collected for the corresponding graphs shown above.

ADDITIONAL METHODS

ELISpot: Enyzme-linked immuno spot (ELISpot) assay was performed using Mabtech reagents according to the manufacturer's instructions. Briefly, splenocytes harvested from immunized mice were subjected to red blood cell lysis and plated on plates (Millipore Multiscreen MSIPN3W) pre-coated with anti-mouse IFNγ Ab (AN16). Samples were plated in triplicate and cells were incubated for 18h +/- 5μM peptide, washed and then incubated with biotinylated detection Ab (R4-6A2). Following incubation with Streptavidin-ALP, substrate solution was added and color development stopped when spots appeared. Plates were analyzed in an Immunospot reader (CTL Europe).

Additional File 5
(Associated with Fig. 2)

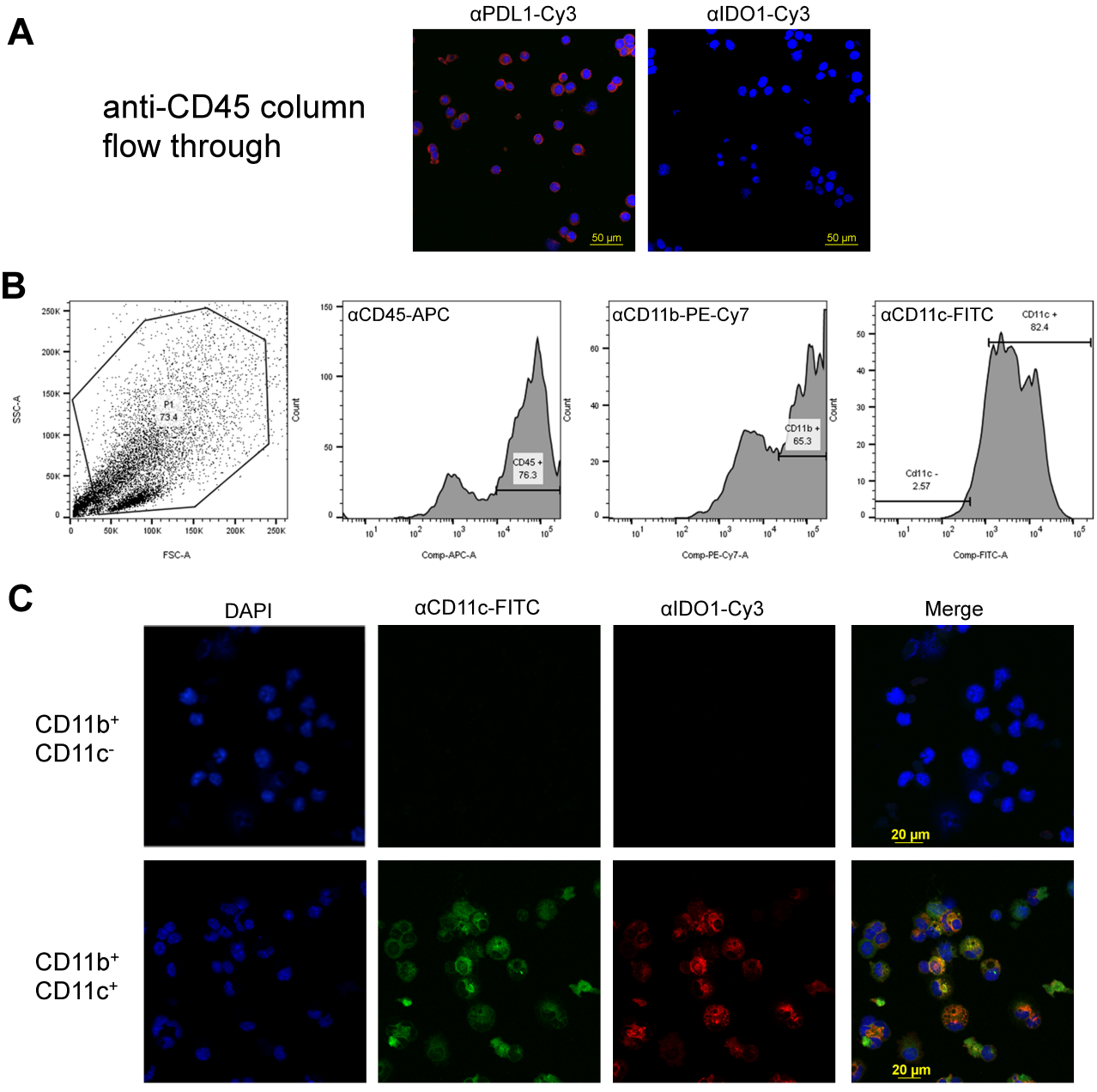


Additional File 5. IFNγ induces PDL1 but not IDO1 expression in CT26 tumor cells *in vitro*. (**A**) Confocal images of cultured CT26 cells ± IFNγ stimulation stained with: *top row* anti-PDL1-Cy3 and DAPI, *bottom row* anti-IDO1-Cy3 and DAPI. (**B**) Confocal images of cultured IDO1-inducible Trex cells ± doxycycline stimulation stained with anti-IDO1-Cy3 and DAPI.

ADDITIONAL METHODS

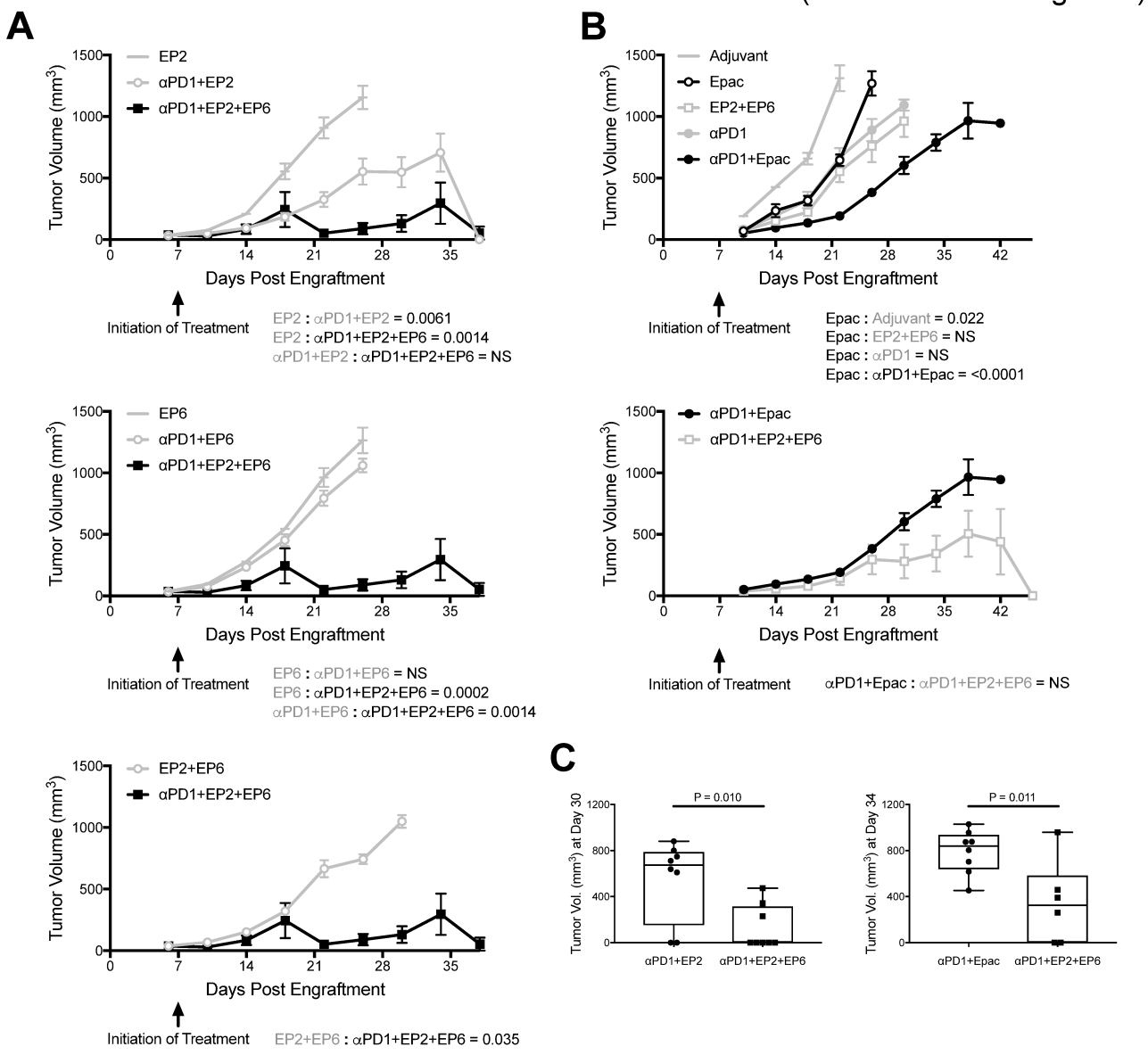
In Vitro Analysis of IDO1 Expression: Comparative inducibility of IDO1 and PDL1 in CT26 tumor cells *in vitro* was performed by culturing the cells in DMEM media supplemented with 100 ng/ml mouse IFNɣ (BD Biosciences) and incubating for 72 hours. The T-Rex™-293 cell line expressing doxycycline inducible mouse IDO1 was stimulated with or without 1µg/ml doxycycline for 24 hours. To prepare samples for confocal microscopy, cells were trypsinized and adhered onto a slide using a Shandon cytospin 3 machine at 800 rpm. Immunofluorescence analysis to detect the cellular expression of IDO1 and PDL1 was performed using the following antibodies: anti-mouse IDO1 (clone 4B7; Millipore), biotin anti-mouse PDL1 (clone 10F.9G2; Biolegend).

Additional File 6
(Associated with Fig. 2)



Additional File 6. Enrichment of the IDO1-expressing immune cells from CT26 tumors established in a WT host. (**A**) An anti-CD45 magnetic bead column was used to positively select out immune cells from tumor cells. Cells in the flow through were stained for nuclei (DAPI) and either PD-L1 (Cy3, red) or IDO1 (Cy3, red) (**B**) Flow cytometry gating parameters for sorting. *left to right* Cells were sequentially gated on forward/side scatter, and positive staining for CD45 and CD11b. The positive and negative CD11c populations were collected. (**C**) Confocal images of the flow cytometry sorted cells. *top row* $CD45^+$, $CD11b^+$, $CD11c^-$ cells, *bottom row* $CD45^+$, $CD11b^+$, $CD11c^+$ cells. *left to right* Immunofluoresence imaging of nuclei (DAPI, blue), CD11c (FITC, green), IDO1 (Cy3, red), and the composite image.

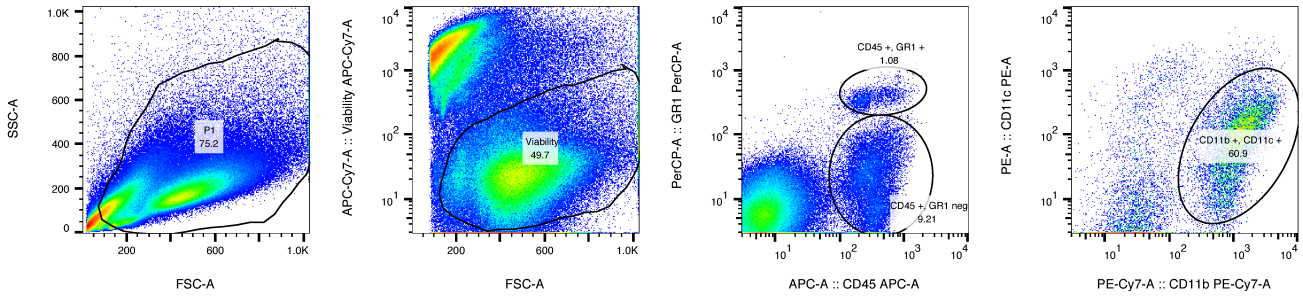
Additional File 7
(Associated with Figure 4)



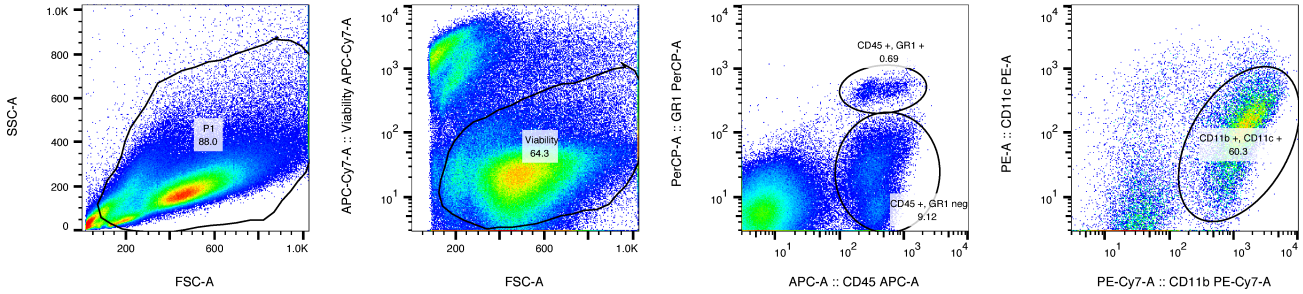
Additional File 7. Cooperativity of anti-PD1 antibody with MHC class I and II-directed IDO1 peptides compared with epacadostat. (**A,B**) Graphs of select CT26 tumor growth curves from the Fig. 4A and 4B datasets respectively to highlight comparisons between the indicated treatment groups. *p*-values for longitudinal tumor growth comparisons between treatment groups are indicated on each graph. (**C**) Although not meeting the threshold for significance in these experiments, the growth curve differentials observed between the anti-PD1+EP2 and anti-PD1+EP2+EP6 groups and the anti-PD1+Epac and anti-PD1+EP2+EP6 groups were reproducible in repeat experiments (data not shown) and were significantly different at specific time points during the latter stage of tumor growth as exemplified in the two graphs.

Additional File 8
(Associated with Figure 5)

Adjuvant

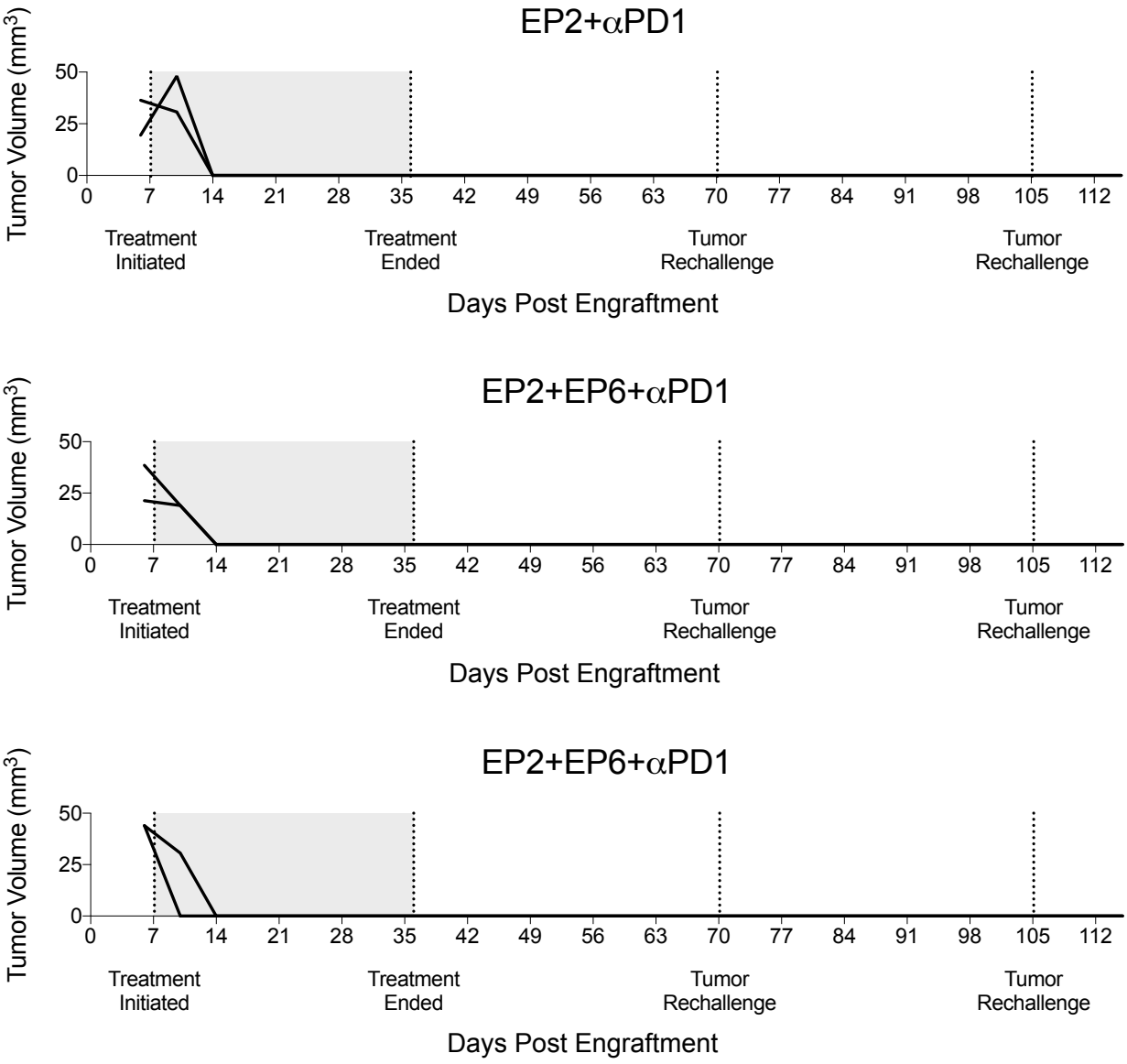


EP2+EP6



Additional File 8. Representative flow cytometry plots of dissociated CT26 tumors from mice treated with adjuvant alone (top) or the EP2+EP6 IDO1 peptides (bottom) for quantitative assessment of the IDO1-expressing, $CD45^{+}$ $Gr1^{-}$ $CD11b^{+}$ $CD11c^{+}$ infiltrating immune cell population. Sequential gating of selected cell populations is shown from left to right for forward scatter vs. side scatter, forward scatter vs viability, CD45 vs. Gr1 and CD11b vs CD11c. Percent of parent population is indicated within each of the gates.

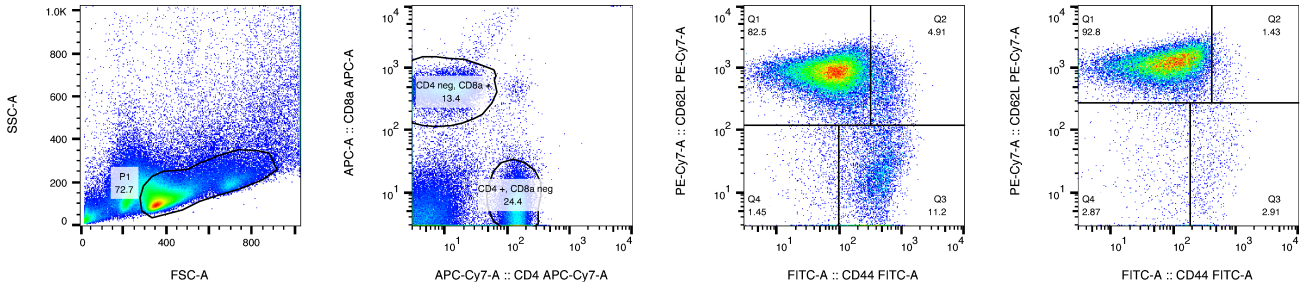
Additional File 9
(Associated with Fig. 4)



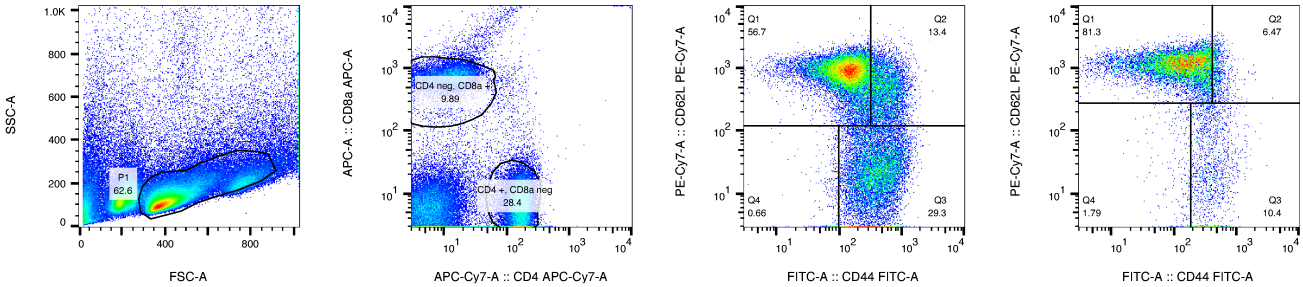
Additional File 9. Mice with complete tumor regressions exhibited durable responses and resistance to subsequent tumor rechallenge. Individual tumor growth curves from among the data sets shown in Fig. 5A where complete regressions were observed in response to treatment with peptide vaccine (either EP2 or EP2+EP6) and anti-PD1 antibody as indicated. The timeline in days shown on the X axis begins with the initial tumor cell engraftment at day 0 followed by initiation and cessation of treatment and subsequent rechallenge with tumor cells. 10 days following the second rechallenge, mice were euthanized to obtain the spleen and lymph nodes for adoptive transfer studies.

Additional File 10
(Associated with Fig. 6)

Naive

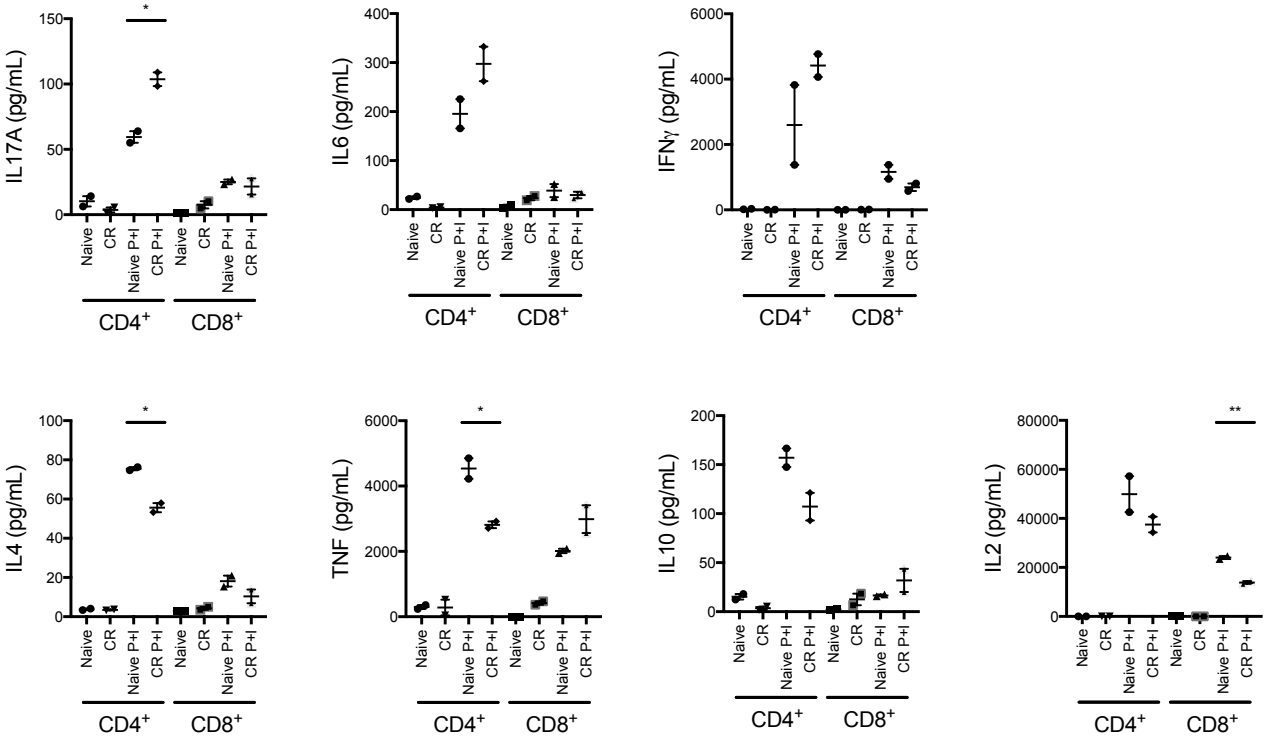


Complete Responder



Additional File 10. Representative flow cytometry plots of dissociated splenocytes for examination of T cells from either untreated naïve mice (top) or mice exhibiting complete tumor responses following treatment with both the EP2 and EP6 IDO1 peptides + anti-PD1 antibody (bottom). Gating of selected cell populations is shown sequentially from left to right based on forward scatter vs. side scatter and CD4 (APC-Cy7) vs. CD8 (APC). The gated $CD4^+$ and $CD8^+$ populations were evaluated for CD44 (FITC) vs.CD62L (PE-Cy7) on the adjacent two plots. Activated T cells were identified as $CD44^{hi}$ $CD62L^{hi}$ (Q2) and memory T cells were identified as $CD44^{hi}$ $CD62L^{lo}$ (Q3). Percent of parent population is indicated within each of the gates.

Additional File 11
(Associated with Fig. 6)



Additional File 11. Cytokine profiling of T cells from complete responder animals. Cytokine levels were measured by cytokine bead array analysis of supernatants from $CD4^+$ and $CD8^+$ splenocytes isolated by flow cytometry and cultured overnight without or with PMA+ionomycin. Splenocytes were obtained from both naïve mice and mice previously exhibiting complete tumor responses following treatment with both the EP2 and EP6 IDO1 peptides + anti-PD1 antibody. Graphed as means ± SEM with significance determined by 2-tailed Student's t-test (*N* = 2 mice/cohort).

ADDITIONAL METHODS

Cytokine Bead Array Analysis: $CD4^+$ and $CD8^+$ splenocytes isolated from naïve and complete responder mice were incubated in RPMI 1640 for 24 hours in a V bottomed 96 well tissue culture plate (Corning) with either DMSO or combination of PMA (50 ng/ml) and ionomycin (500 ng/ml). Cytokine levels in the culture media were measured using the BD™ Mouse Th1/Th2/Th17 Cytometric Bead Array Kit (BD biosciences) as per the manufacturer's instructions. FACS Canto flow cytometer (BD Biosciences) and FACSDIVA software (BD Biosciences) was used to read the samples and FCAP array software was used to determine the cytokine concentrations.