SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1. AD5-10 only binds to DR5 without cross-reacting with other death receptors. *A,* expression of death receptors on THP-1 cells, Jurkat cells and NIH3T3 cells. Cells were probed with respective antibodies followed by flow cytometry assay. *B,* AD5-10 binds to wild-type human membrane DR5 on cells. Cells were probed AD5-10 or normal mouse IgG_3 isotype control and TRITC-labeled secondary antibody followed by flow cytometry assay.

FIGURE S2. Binding affinity of AD5-10, Adie-1 and Adie-2 to the synthetic peptides. Binding affinity of Ad5-10, Adie-1 and Adie-2 to synthetic wild-type epitope 1 (*A*), wild-type epitope 2 (*B*), immunizing epitope (*C*), mutant epitope 1 (*D*), mutant epitope 2 (*E*), N1 cap (*F*), CRD1A (*G*) and random peptide (*H*). Synthetic peptides were immobilized on a 96-well plate. The plate was then incubated with the indicated concentration of AD5-10, Adie-1 and Adie-2. Then, the binding affinities were measured by ELISA. The absorbance was measured at 450 nm on a microplate reader. Values represent the mean ± SD of triplicate samples.

FIGURE S3. AD5-10 and TRAIL-induced DISC in Jurkat cells. Analysis of AD5-10-induced DISC (left panel) and TRAIL-induced DISC (right panel) in Jurkat cells. For AD5-10-induced DISC analysis, AD5-10 was added to the cells either after in cell lysis in unstimulated control cells or for 60 min before cell lysis in stimulated cells. For TRAIL-induced DISC analysis, TRAIL-FLAG and anti-FLAG were added to the cells either after in cell lysis in unstimulated control cells or for 60 min before cell lysis in stimulated cells. All the samples were immunoprecipitated with protein G-Sepharose 4B and analyzed by immunoblotting. Whole cell extracts (WCE) were also analyzed on the WB for the presence of the proteins.

FIGURE S4. Antibodies against NTR of DR5 can induce weak/moderate cell death in NSCLC cells. *A,* Hoechst 33258 staining. H460 cells were cultured with 100 ng/ml anti-DR5 mAbs or rsTRAIL for 24 h. After fixation, cells were stained with Hoechst 33258 (1 μg/m) and nuclear condensation and fragmentation of chromatin were observed by fluorescence microscopy (20×). The excitation wavelength was 380 nm. *B,* H460 cells are more sensitive to TRAIL than to anti-DR5 mAbs through cleavage of c-$FLIP_L$ and activation of caspase-3. H460 cells were incubated with anti-DR5 mAbs or 100 ng/ml rsTRAIL for 24 hrs. The cleavage of c-$FLIP_L$ and activation of caspase-3 were tested using the respective antibodies.

Figure S1

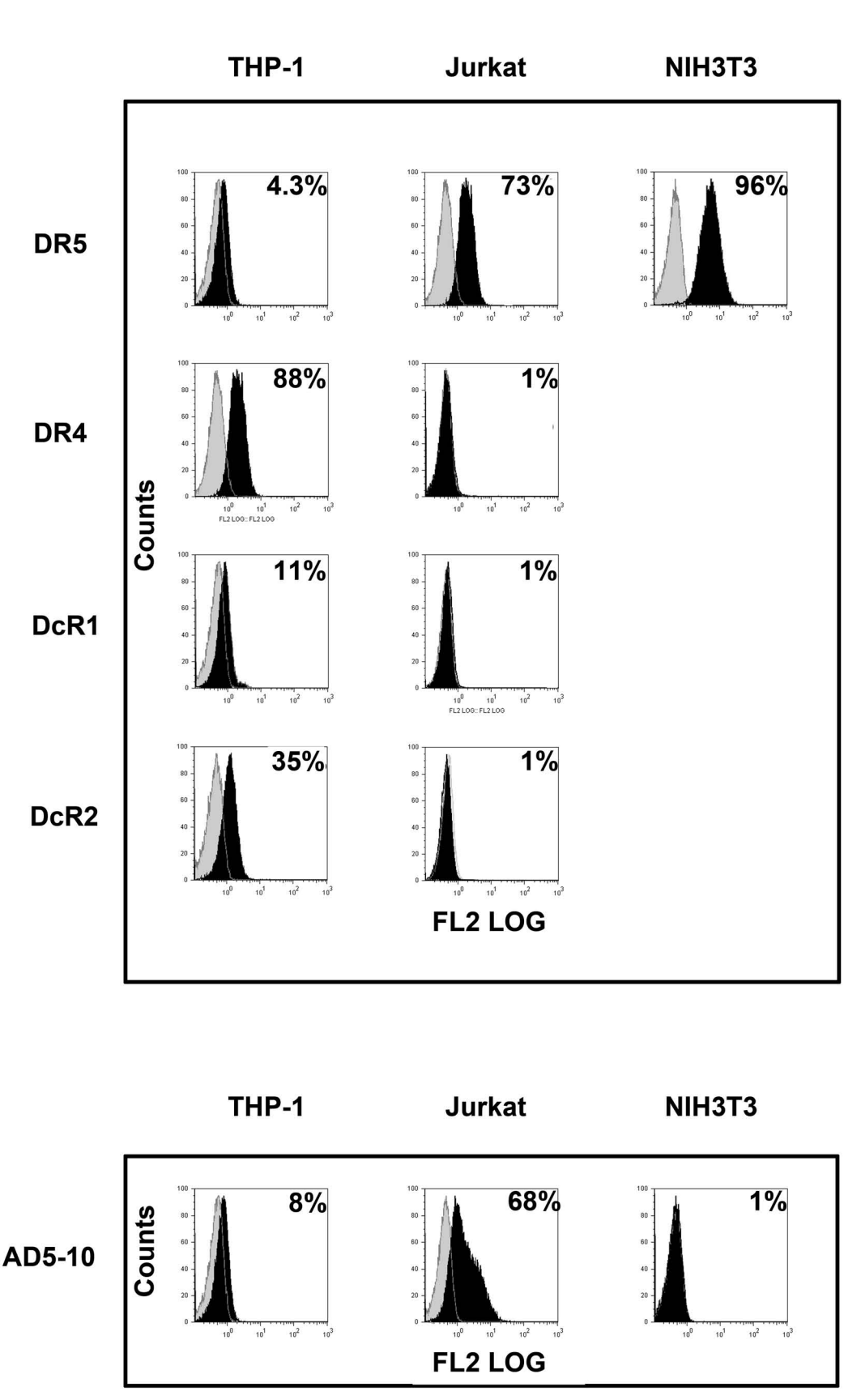

A
THP-1
Jurkat
NIH3T3
DR5
DR4
DcR1
DcR2
4.3%
73%
96%
88%
1%
11%
1%
35%
1%
Counts
FL2 LOG
B
THP-1
Jurkat
NIH3T3
AD5-10
8%
68%
1%
Counts
FL2 LOG

Figure S2

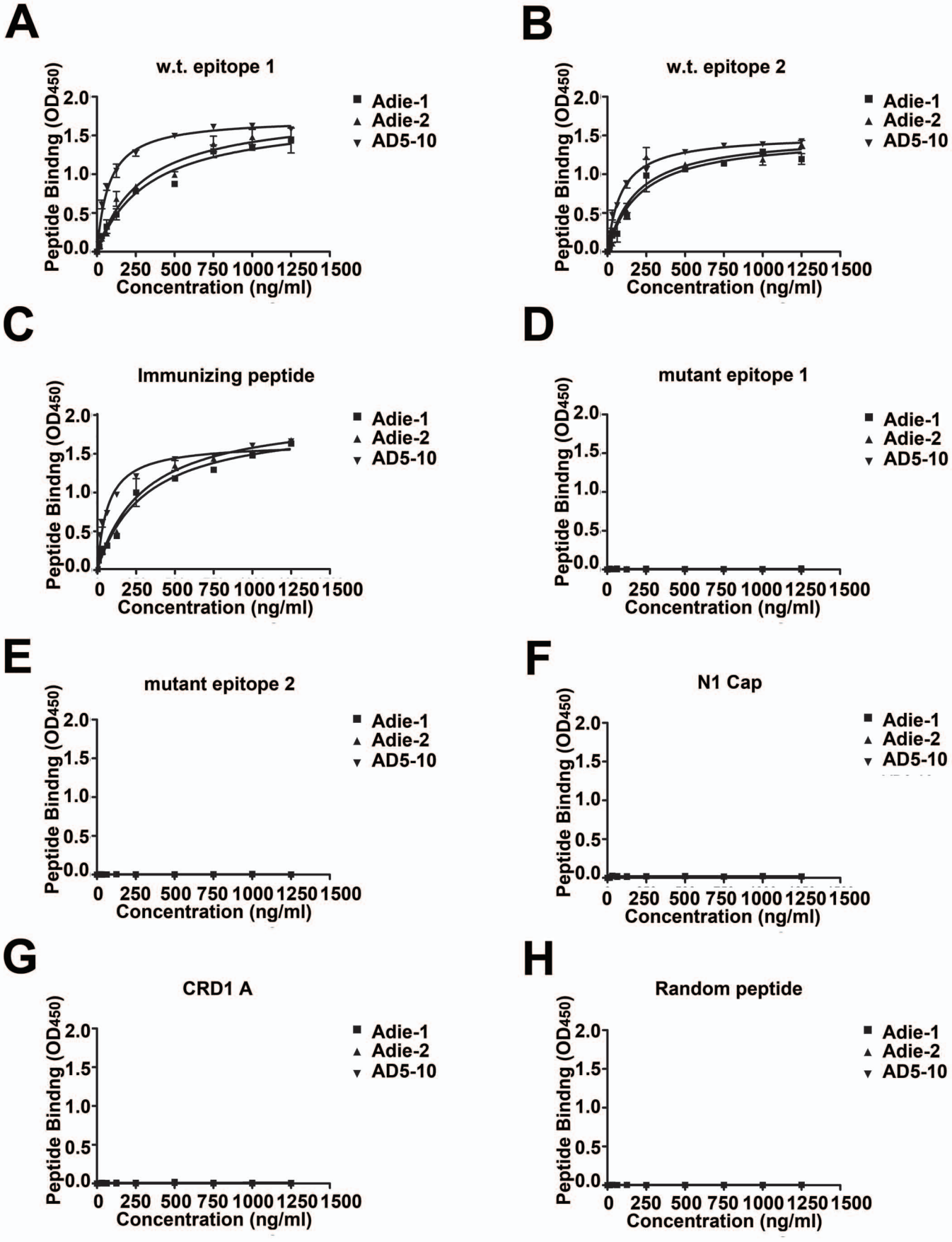

A
w.t. epitope 1
B
w.t. epitope 2
C
Immunizing peptide
D
mutant epitope 1
E
mutant epitope 2
F
N1 Cap
G
CRD1 A
H
Random peptide
Peptide Bindng (OD450)
Concentration (ng/ml)
0 250 500 750 1000 1250 1500
0.0 0.5 1.0 1.5 2.0
Adie-1
Adie-2
AD5-10

Figure S3

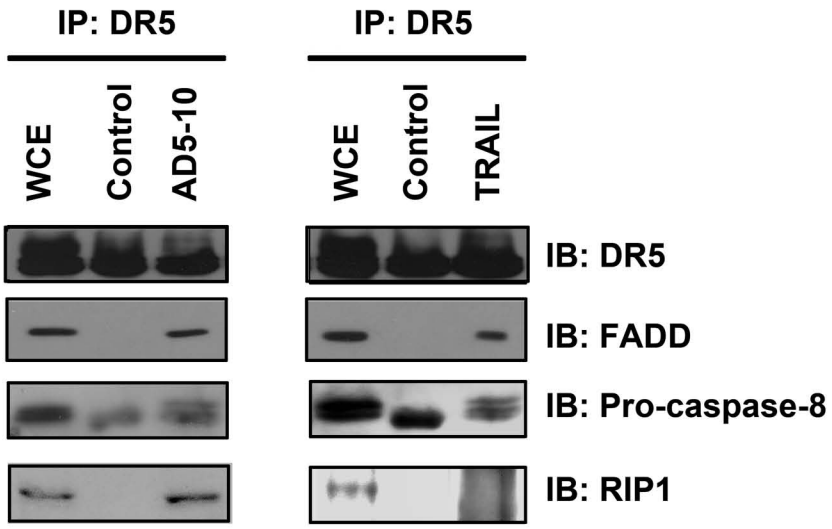
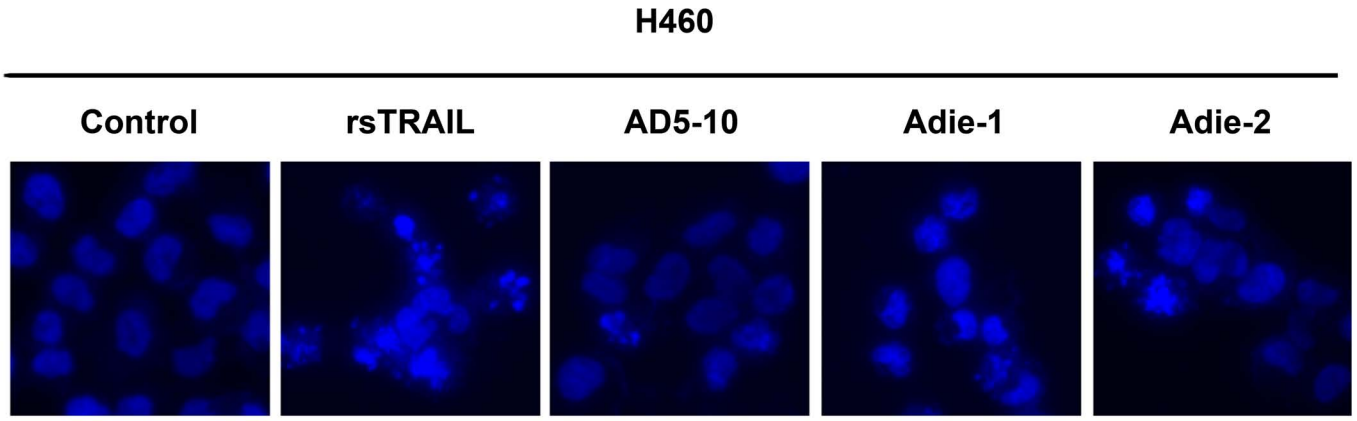
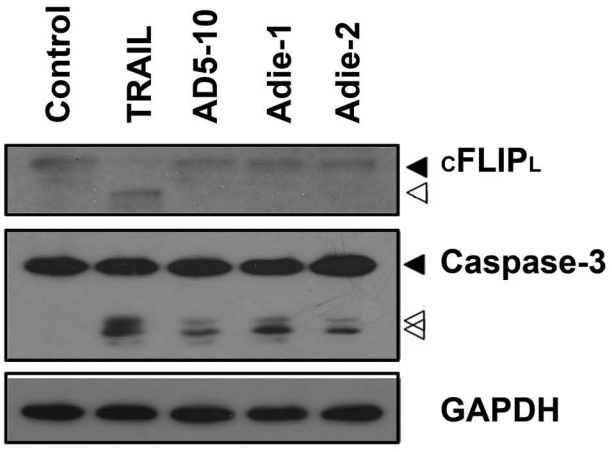

IP: DR5
IP: DR5
WCE
Control
AD5-10
WCE
Control
TRAIL
IB: DR5
IB: FADD
IB: Pro-caspase-8
IB: RIP1

Figure S4

A


H460
Control
rsTRAIL
AD5-10
Adie-1
Adie-2

B


Control
TRAIL
AD5-10
Adie-1
Adie-2
cFLIPL
Caspase-3
GAPDH