Supporting information (SI)

Adamantyl isothiocyanates as mutant p53 rescuing agents and its structure-activity relationships

Vladimir Burmistrov[1,2*], Rahul Saxena[3], Dmitry Pitushkin[1], Gennady M. Butov[1,2], Fung-Lung Chung[4] and Monika Aggarwal[4*#]

[1] Volgograd State Technical University, Volgograd, 400005, Russia, [2] Department of Chemistry, Technology and Equipment of Chemical Industry, Volzhsky Polytechnic Institute (branch) Volgograd State Technical University, Volzhsky, 404121, Russia, [3]Department of Biochemistry and Molecular and Cellular Biology, Georgetown University, Washington, DC 20007, [4]Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University, Washington DC 20007, USA

*These authors contributed equally to this work

[#]Corresponding author

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Supplementary Figures and Figure legends

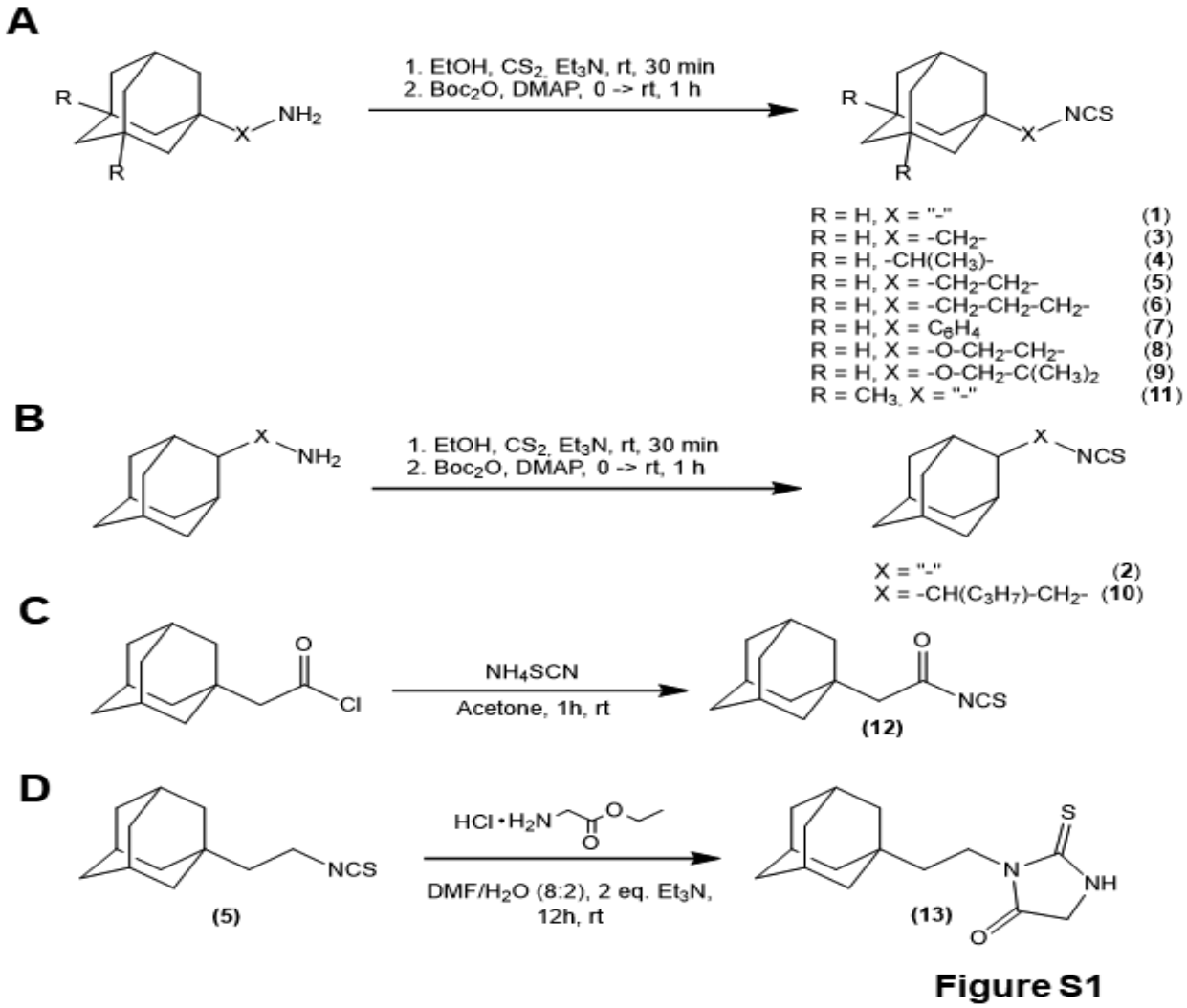

Figure S1. Scheme for the synthesis of Ad-ITCs 1-12 **and Ad-compound** 13**. (A)** Scheme for the synthesis of Ad-ITCs **1**, **3**, **4**, **5**, **6**, **7**, **8**, **9** and **11**. (**B**) Scheme for the synthesis of Ad-ITCs **2** and **10**. (**C**) Scheme for the synthesis of Ad-ITC **12**. (**D**) Scheme for the synthesis of Ad-compound **13**.

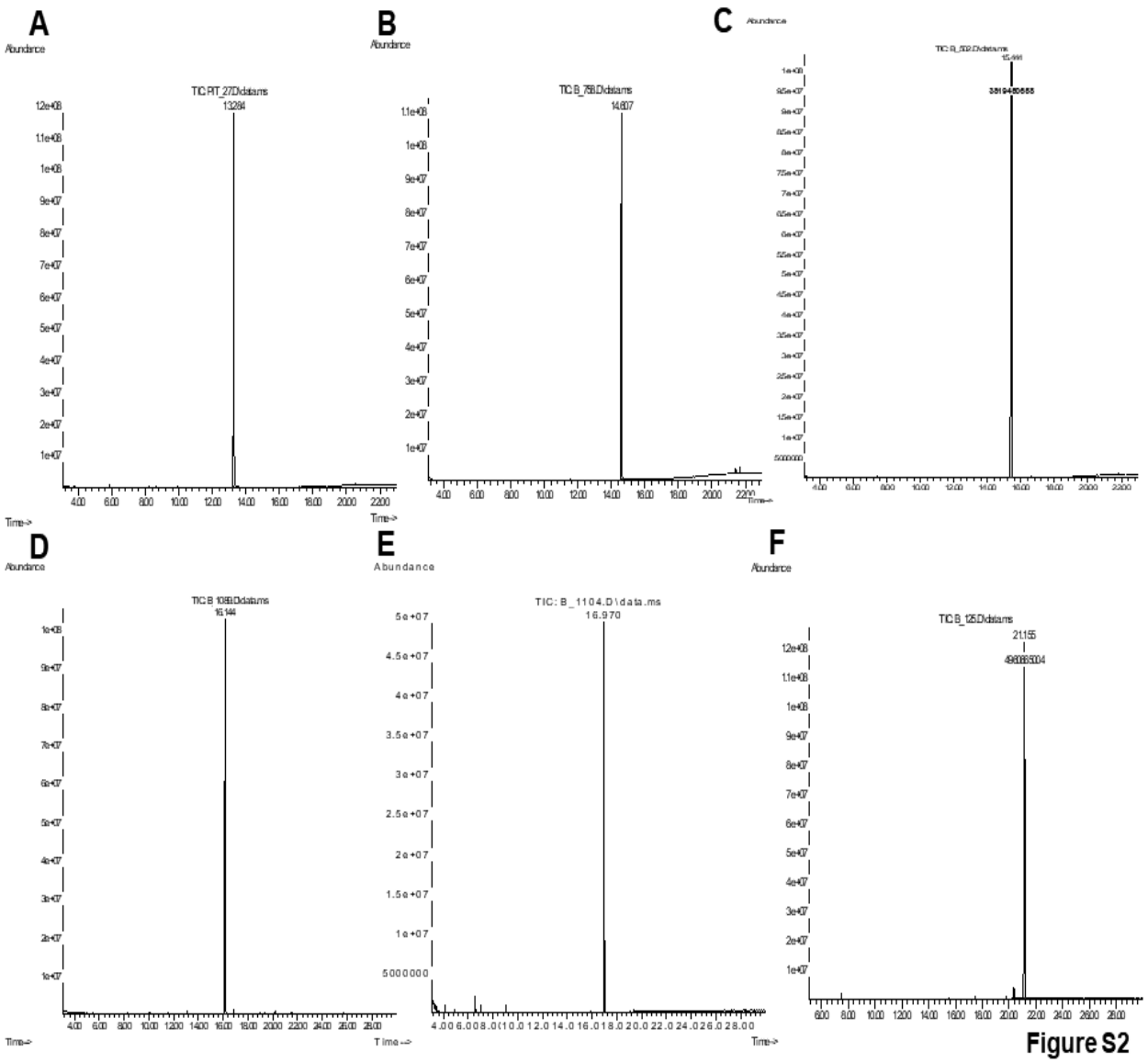

Figure S2. Purification peaks and percentage purity of Ad-ITCs 1 **and** 3-7 **as determined by GC-MS. 1**, 99.8% (**A**), **3**, 99.0% (**B**), **4**, 99.7% (**C**), **5**, 99.5% (**D**), **6**, 98.4% (**E**) and **7**, 98.0% (**F**).

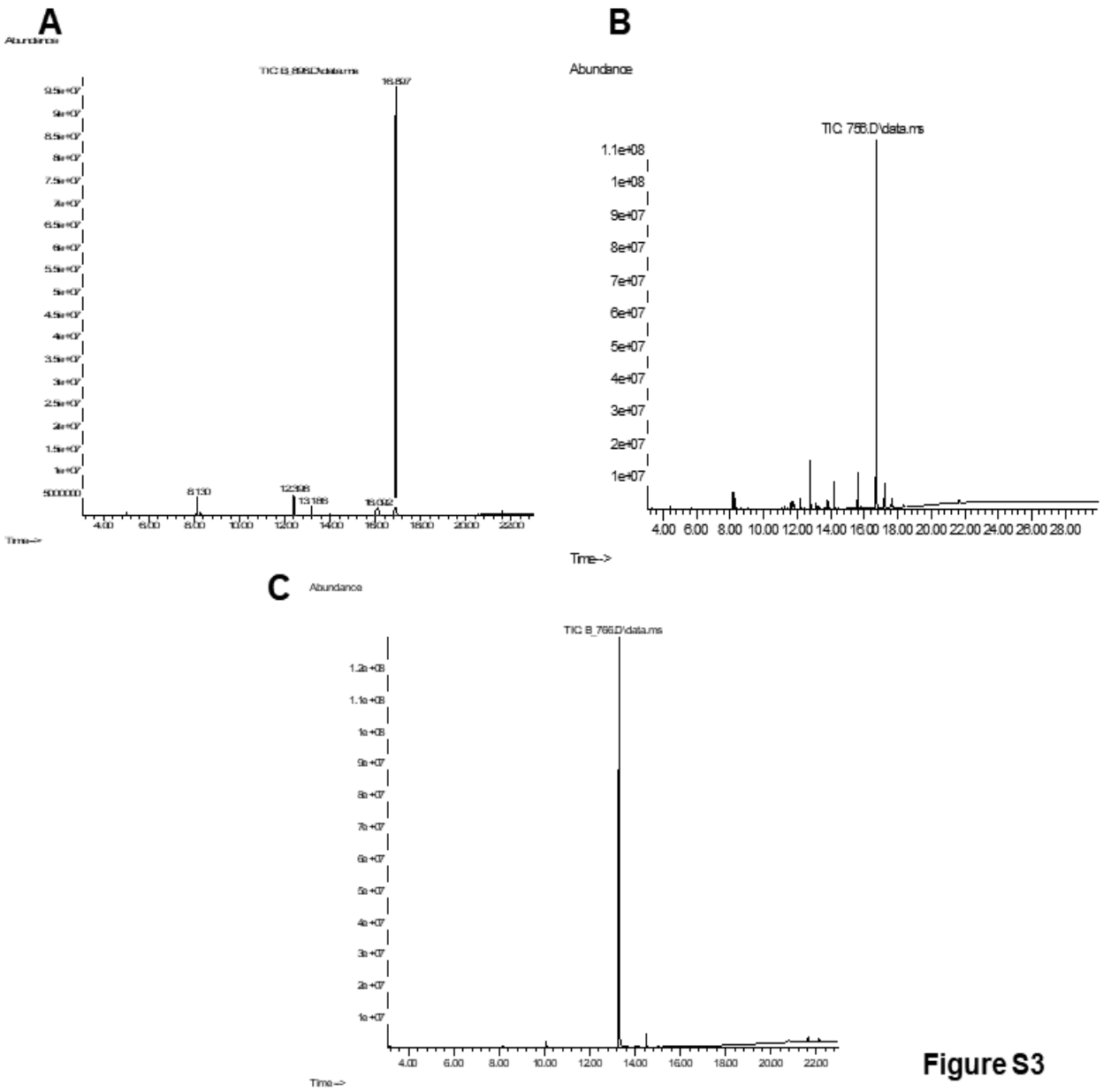

Figure S3

Figure S3. Purification peaks and percentage purity of Ad-ITCs 8**,** 9 **and** 11 **as determined by GC-MS. 8**, 97.5% (**A**), **9**, 95.9% (**B**) and **11**, 99.2% (**C**).

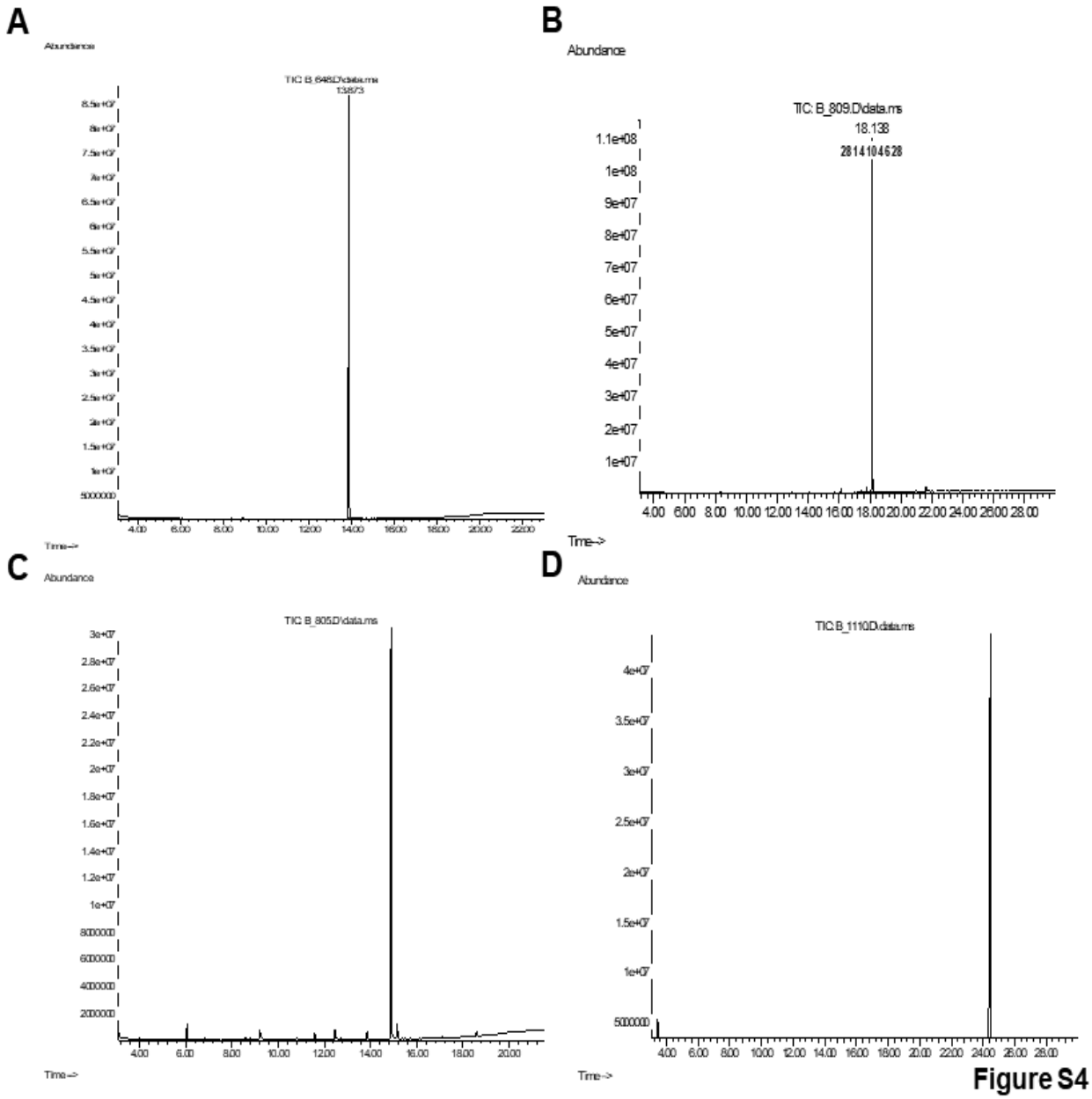

Figure S4. Purification peaks and percentage purity of Ad-ITCs 2**,** 10 **and** 12 **and Ad-compound** 13 **as determined by GC-MS. 2**, 99.8% (**A**), **10**, 99.5% (**B**), **12**, 98.2% (**C**) and **13**, 99.5% (**D**).

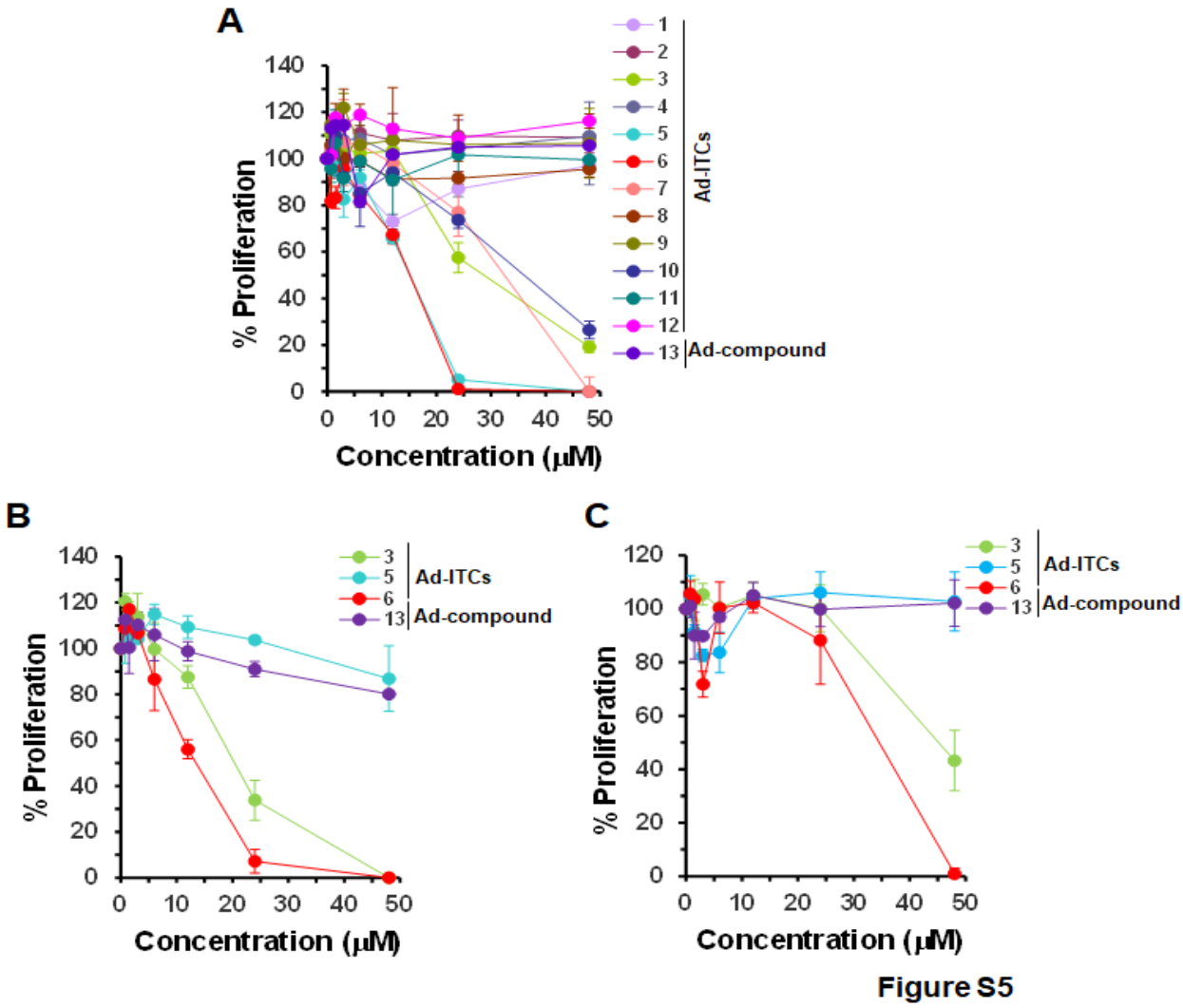

Figure S5. Effects of Ad-ITCs on the proliferation of breast cancer cell lines expressing mutant p53 or WT p53. (**A-C)** The mutant p53 ($p53^{R280K}$ MDA-MB-231 and $p53^{R273H}$ MDA-MB-468) and WT p53 (MCF-7) cells, respectively, were treated with DMSO (as a control) or different Ad-ITCs or Ad-compound **13** at the indicated concentrations for 72 h. Percent cell proliferation determined by WST-1 was calculated as the ratio of OD_{450} values obtained for cells grown in the presence of the respective Ad-ITC or Ad-compound **13** compared with the presence of DMSO. Experiments were performed in triplicate. Error bars represents SD.

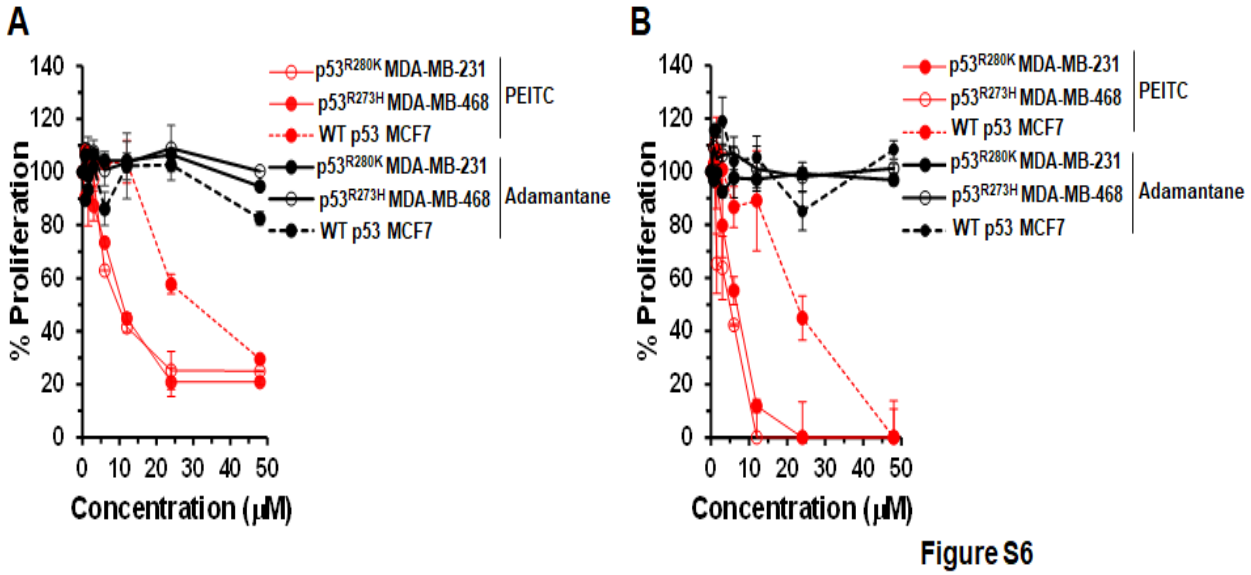

Figure S6. Effects of PEITC and adamantane on the proliferation of breast cancer cell lines expressing mutant p53 or WT p53. The mutant p53 ($p53^{R280K}$ MDA-MB-231 and $p53^{R273H}$ MDA-MB-468) and WT p53 (MCF-7) cells, respectively, were treated with DMSO (as a control), or PEITC or adamantane at the indicated concentrations for 24 h (**A)** or 72 h (**B**), respectively. Percent cell proliferation determined by WST-1 was calculated as the ratio of OD_{450} values obtained for cells grown in the presence of PEITC or adamantane compared with the presence of DMSO. Experiments were performed in triplicate. Error bars represents SD.

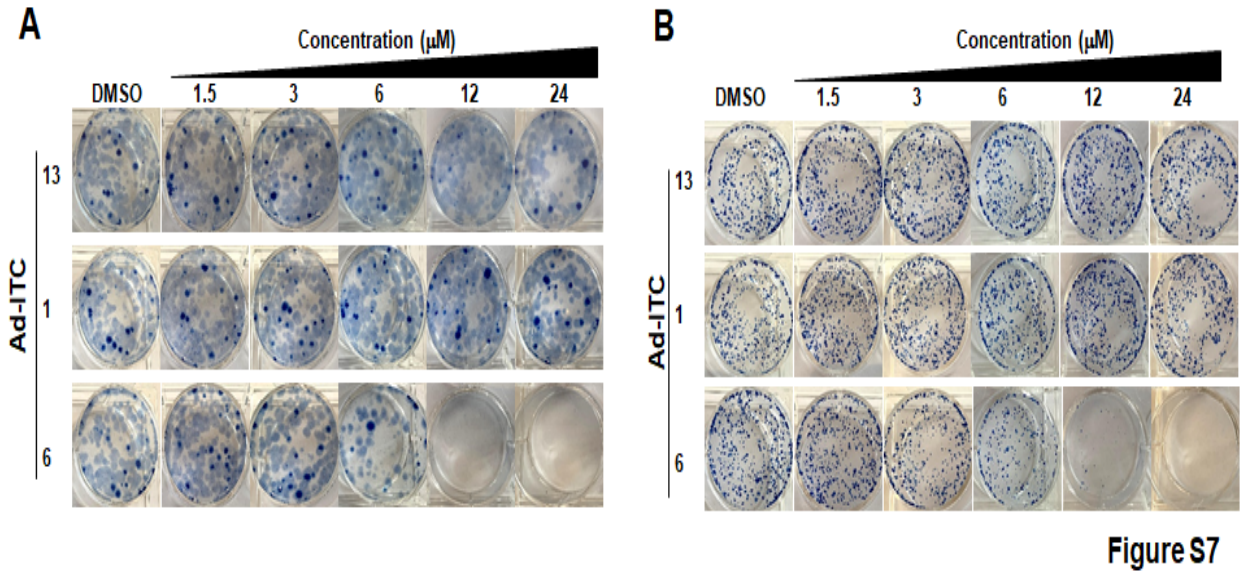

Figure S7. Colony formation assay to determine the effects of Ad-ITCs on the growth of breast cancer cell lines expressing mutant p53 or WT p53. The $p53^{R280K}$ MDA-MB-231 (**A**) and WT p53 (MCF-7) (**B**) cells were treated with DMSO or the indicated concentration of Ad-ITCs or Ad-compound **13** for 24 h. Cells were then allowed to grow in fresh medium lacking DMSO or Ad-ITC or Ad-compound **13** for 10 d and stained with methylene blue. Experiments were performed in triplicate. Error bars represents SD.

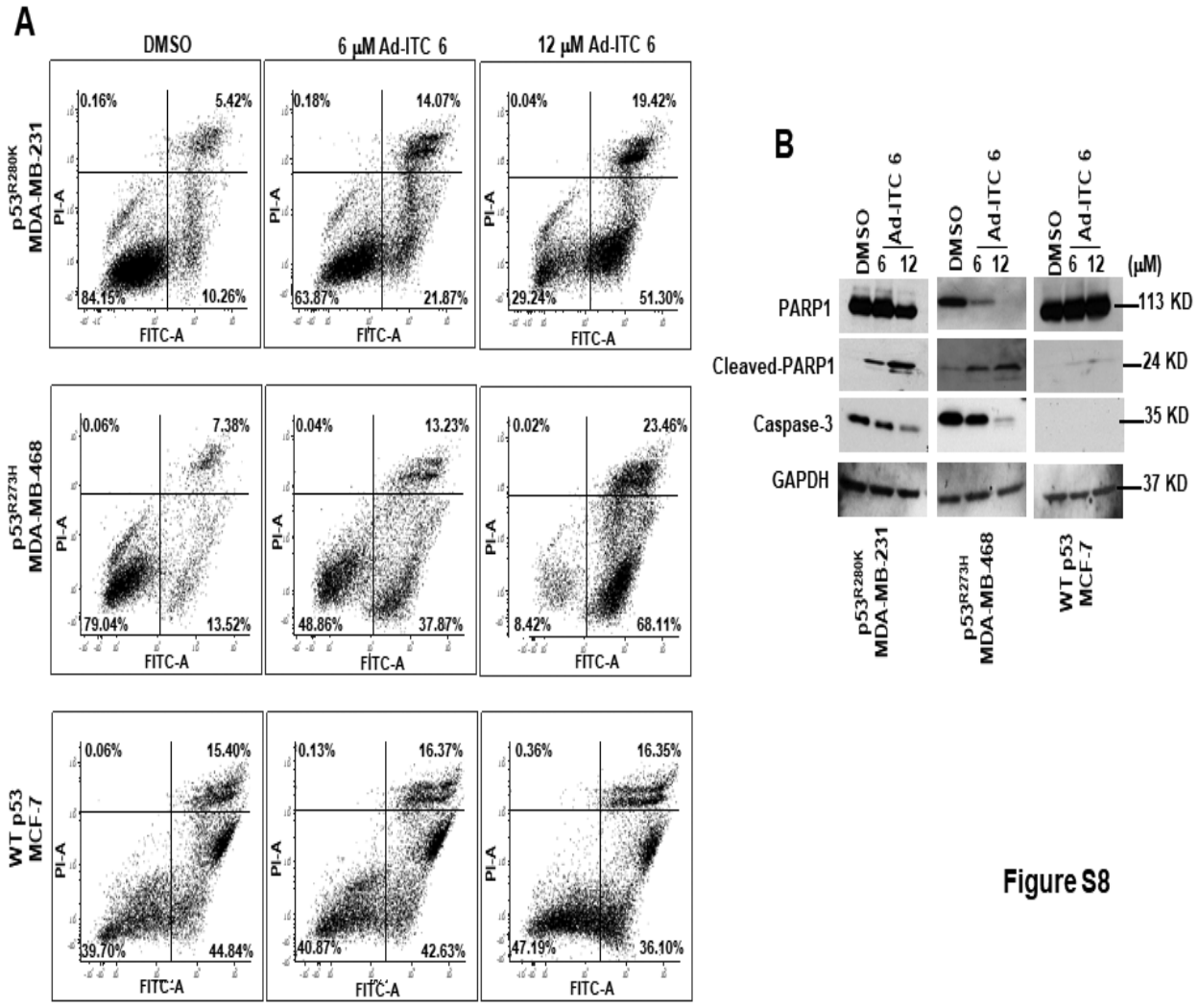

Figure S8

Figure S8. Ad-ITC 6 **induced apoptosis in breast cancer cell lines expressing mutant p53, but not WT p53.** (**A**) The mutant p53 ($p53^{R280K}$ MDA-MB-231 and $p53^{R273H}$ MDA-MB-468) and WT p53 (MCF-7) cells, respectively, were treated with DMSO (as a control) or Ad-ITC **6** at the indicated concentrations for 24 h. Apoptosis was measured by Annexin-V staining by flow cytometry using a BD LSRFORTESSA instrument. Representative FACS analysis plots of cell death measured by flow cytometry through Annexin-V staining. (**B**) Effects of Ad-ITC **6** on caspase 3 and PARP1 levels. The mutant p53 ($p53^{R280K}$ MDA-MB-231 and p53 ^{R273H} MDA-MB-468) and WT p53 (MCF-7) cells, respectively, were treated with DMSO (as a control) or 6 μM or 12 μM of Ad-ITC **6** for 24 h. Cells were harvested and lysates were prepared. Hundred μg of

the lysate fractions were resolved by SDS-PAGE and probed with anti-PARP or anti-caspase 3 antibodies. Blots were stripped and re-probed with anti-GAPDH as a loading control. Experiments were performed in triplicate.

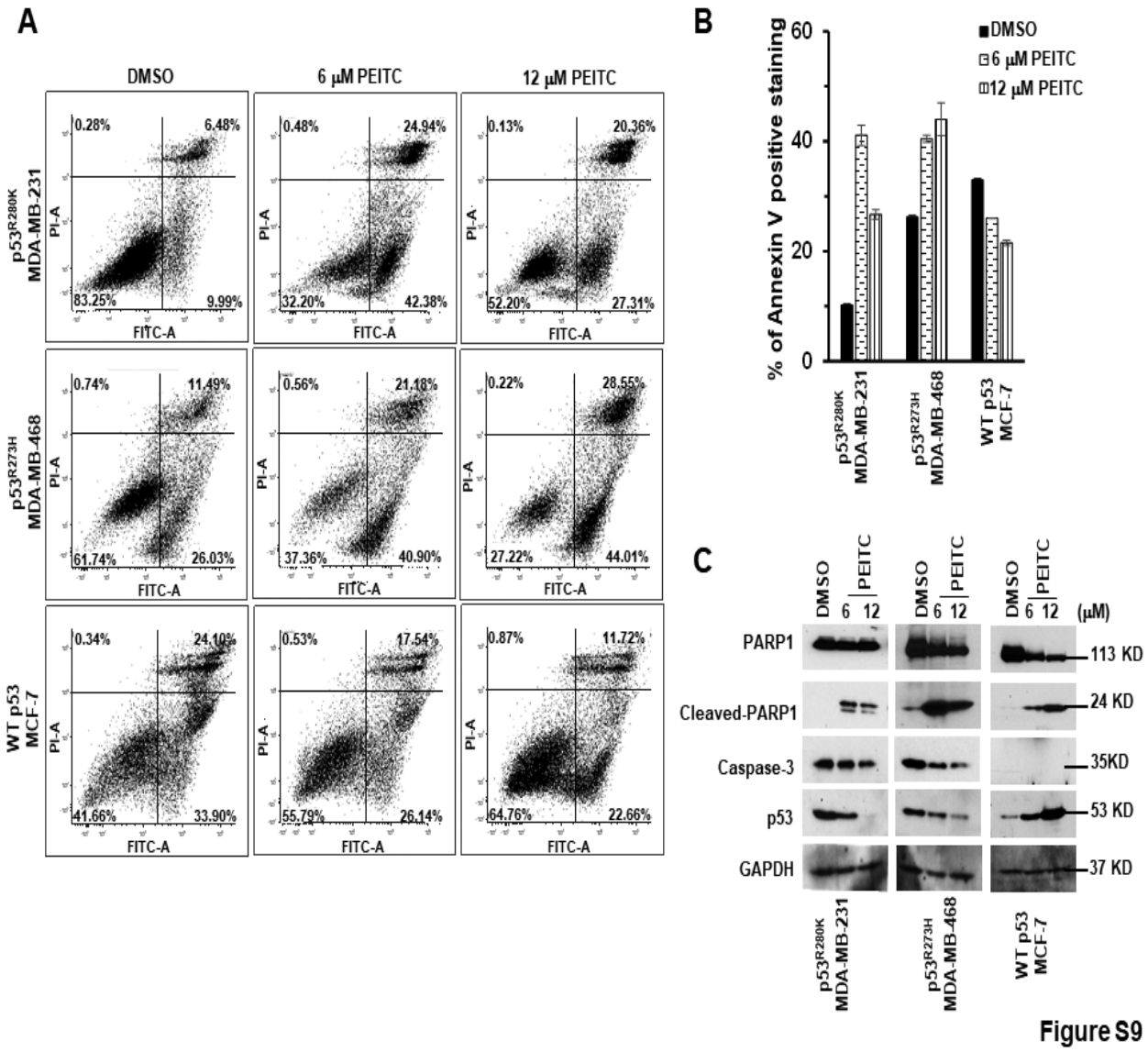

Figure S9. Effects of PEITC on apoptosis induction and p53 levels in breast cancer cell lines with mutant p53 or WT p53. The mutant p53 ($p53^{R280K}$ MDA-MB-231 and $p53^{R273H}$ MDA-MB-468) and WT p53 (MCF-7) cells, respectively, were treated with DMSO (as a control) or PEITC at the indicated concentrations for 24 h. Apoptosis was measured by Annexin-V staining by flow cytometry using a BD LSRFORTESSA instrument. (**A**) Representative FACS analysis plots of cell death measured by flow cytometry through Annexin-V staining, and (**B**) Percent of Annexin-V positive stained cells were calculated as the ratio of stained cells obtained

for cells grown in the presence of the PEITC compared with the presence of DMSO. (**C**) Effects of PEITC on caspase 3 and PARP1 levels. The mutant p53 ($p53^{R280K}$ MDA-MB-231 and $p53^{R273H}$ MDA-MB-468) and WT p53 (MCF-7) cells, respectively, were treated with DMSO (as a control) or 6 μM or 12 μM of PEITC for 24 h. Cells were harvested and lysates were prepared. Hundred μg of the lysate fractions were resolved by SDS-PAGE and probed with anti-PARP or anti-caspase 3 antibodies. Blots were stripped and re-probed with anti-GAPDH as a loading control. Experiments were performed in triplicate. Error bars represents SD.

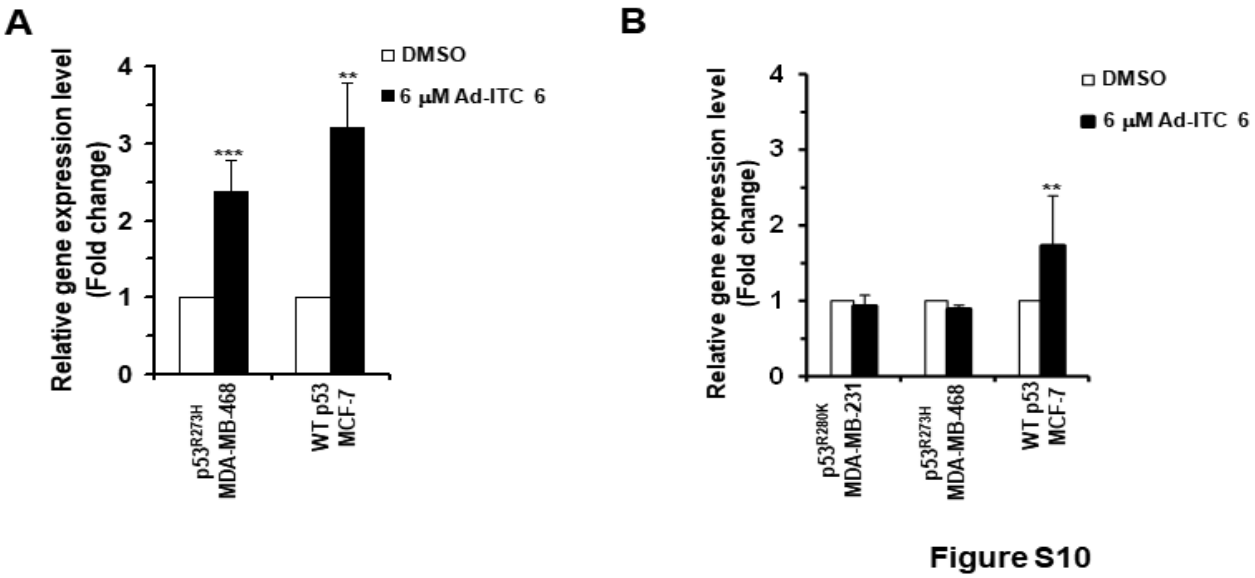

Figure S10. Effects of Ad-ITC 6 **on the expression level of TP53 and its canonical target MDM2.** (**A**) qRT-PCR of p53 regulated downstream target gene MDM2 in mutant $p53^{R273H}$ MDA-MB-468 and WT p53 MCF-7 cell treated with DMSO or 6 μM of Ad-ITC **6** for 4 h. (***p ≤ .0005 and **p ≤ 0.005). (**B**) qRT-PCR of p53 gene in mutant $p53^{R280K}$ MDA-MB-231, $p53^{R273H}$ MDA-MB-468 and WT p53 MCF-7 cell treated with DMSO or 6 μM of Ad-ITC **6** for 4 h. (**p ≤ 0.005). Experiments were performed in triplicate. Error bars represents SD.

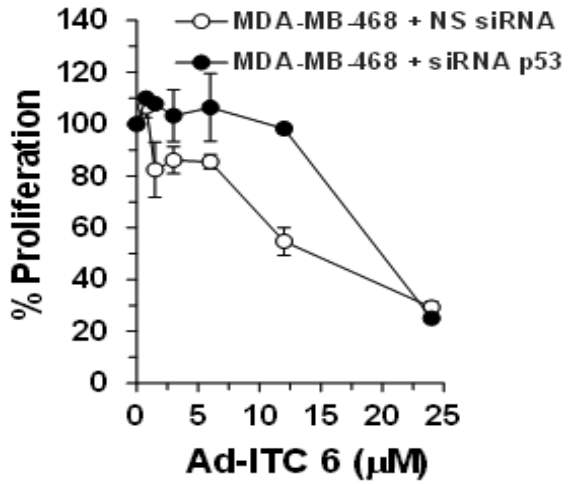

Figure S11

Figure S11. Ad-ITC 6 **inhibits MDA-MB-468 cell proliferation in a mutant p53-dependent manner.** $p53^{R273H}$ MDA-MB-468 cells transfected with non-specific siRNA (NS siRNA) or p53 siRNA were treated with DMSO or Ad-ITC **6** for 24 h. Percent cell proliferation determined by WST-1 was calculated as the ratio of OD_{450} values obtained for cells grown in the presence of the Ad-ITC **6** compared with the presence of DMSO. Experiments were performed in triplicate. Error bars represents SD.

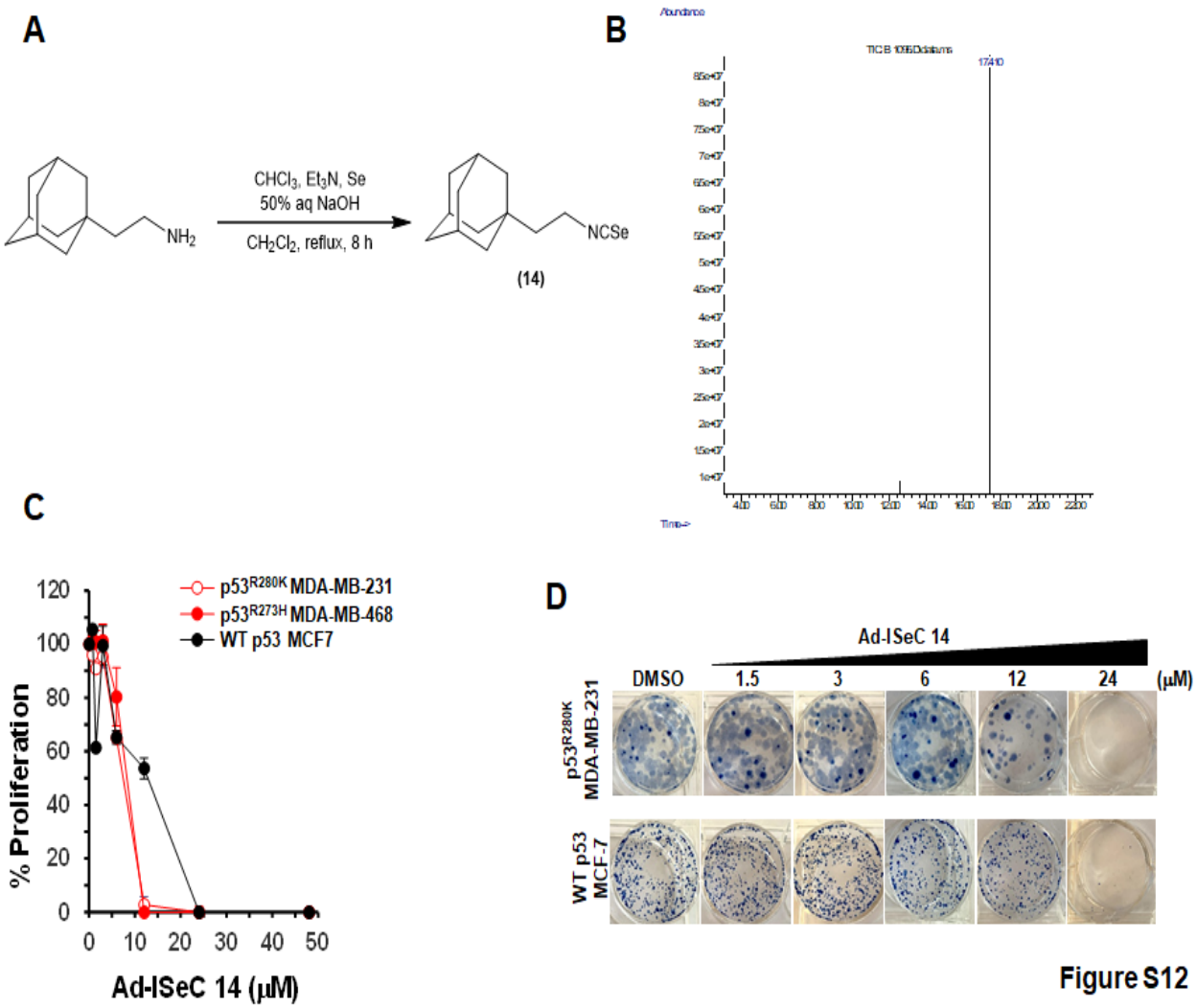

Figure S12. Synthesis and purification peak of Ad-ISeC 14 **and its effects on the proliferation of breast cancer cell lines**. (**A**) Scheme for the synthesis of Ad-ISeC **14**. **(B)** Purification peak of Ad-ISeC **14** as determined by GC-MS. The percentage purity of this compound was 99.2%. (**C**) The mutant p53 ($p53^{R280K}$ MDA-MB-231 and $p53^{R273H}$ MDA-MB-468) and WT p53 (MCF-7) cells, respectively, were treated with DMSO or the indicated concentrations of Ad-ISeC **14** for 72 h. Percent cell proliferation determined by WST-1 was calculated as the ratio of OD_{450} values obtained for cells grown in the presence of the Ad-ISeC **14** compared with the presence of DMSO. (**D**) Effect of Ad-ISeC **14** on $p53^{R280K}$ MDA-MB-231 and MCF-7 cell growth. MDA-MB-231 and MCF-7 cells were treated with DMSO or the

indicated concentration of Ad-ISeC **14** for 24 h. Cells were then allowed to grow in fresh medium lacking DMSO or Ad-ISeC **14** for 10 d and stained with methylene blue. Experiments were performed in triplicate. Error bars represents SD.

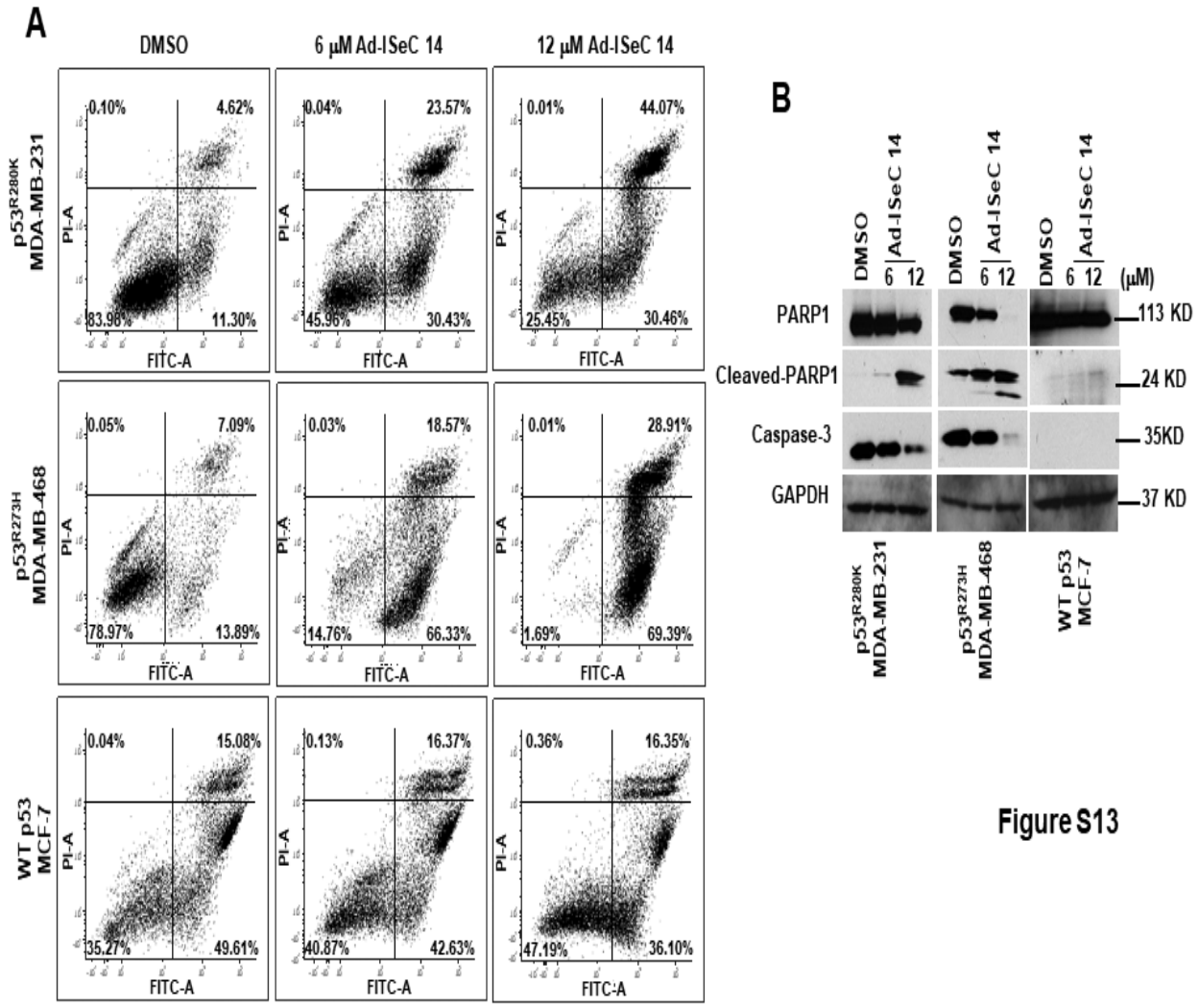

Figure S13

Figure S13. Ad-ITC 14 **induced apoptosis in breast cancer cell lines expressing mutant p53, but not WT p53.** (**A**) The mutant p53 ($p53^{R280K}$ MDA-MB-231 and $p53^{R273H}$ MDA-MB-468) and WT p53 (MCF-7) cells, respectively, were treated with DMSO (as a control) or Ad-ISeC **14** at the indicated concentrations for 24 h. Apoptosis was measured by Annexin-V staining by flow cytometry using a BD LSRFORTESSA instrument. Representative FACS analysis plots of cell death measured by flow cytometry through Annexin-V staining. (**B**) Effects of Ad-ISeC **14** on caspase 3 and PARP1 levels. The mutant p53 ($p53^{R280K}$ MDA-MB-231 and $p53^{R273H}$ MDA-MB-468) and WT p53 (MCF-7) cells, respectively, were treated with DMSO (as a control) or 6 μM or 12 μM of Ad-ISeC **14** for 24 h. Cells were harvested and lysates were prepared. Hundred μg

of the lysate fractions were resolved by SDS-PAGE and probed with anti-PARP or anti-caspase 3 antibodies. Blots were stripped and re-probed with anti-GAPDH as a loading control. Experiments were performed in triplicate.

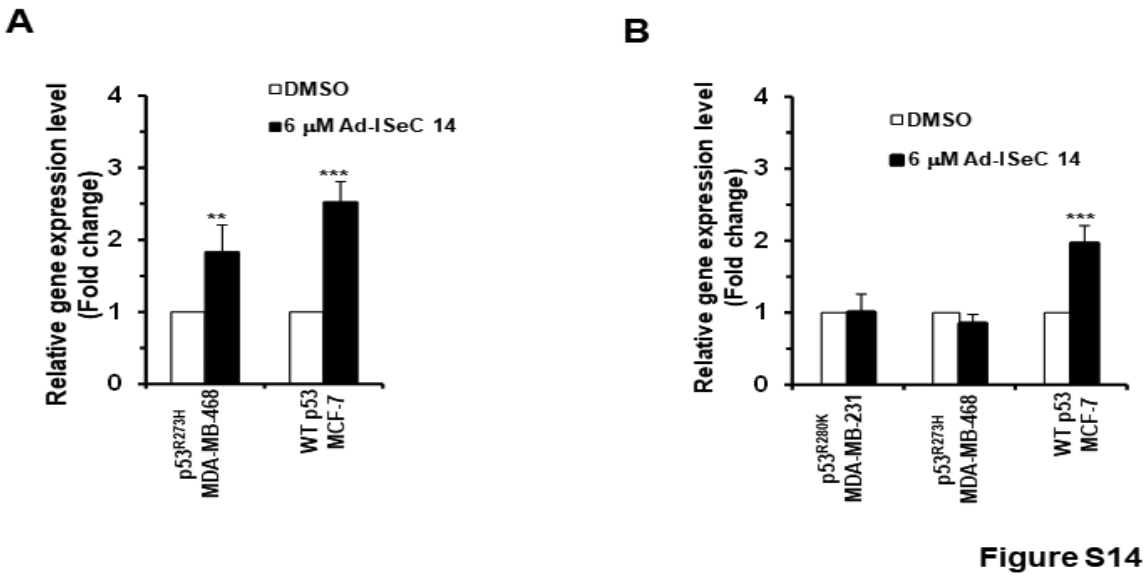

Figure S14. Effects of Ad-ISeC 14 **on the regulation of TP53 and its canonical target MDM2 in breast cancer cell lines.** (**A**) qRT-PCR of p53 regulated downstream target gene MDM2 in mutant $p53^{R273H}$ MDA-MB-468 and WT p53 MCF-7 cell treated with DMSO or 6 μM of Ad-ISeC **14** for 4 h. (***$p \leq .0005$ and **$p \leq 0.005$). (**B**) qRT-PCR of TP53 gene in mutant $p53^{R280K}$ MDA-MB-231, $p53^{R273H}$ MDA-MB-468 and WT p53 MCF-7 cell treated with DMSO or 6 μM of Ad-ISeC **14** for 4 h. (***$p \leq 0.0005$). Experiments were performed in triplicate. Error bars represents SD.

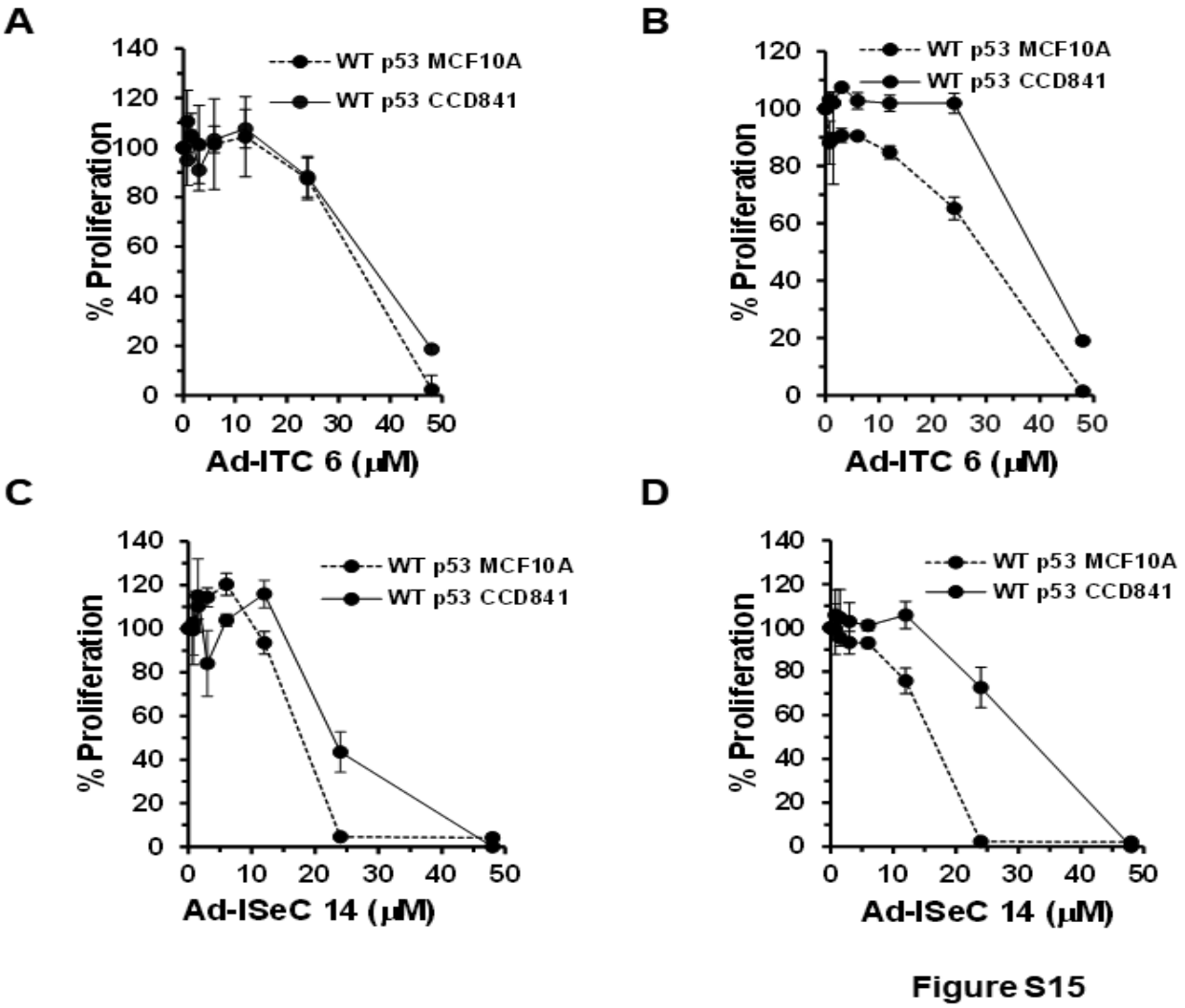

Figure S15. Effects of Ad-ITC 6 **or Ad-ISeC** 14 **on the proliferation of normal mammary epithelial cells or normal colon cells expressing WT p53.** (**A**, **B**) The normal mammary epithelial cells (WT p53 MCF10A) or normal colon cells (WT p53 CCD841), respectively, were treated with DMSO (as a control), or Ad-ITC **6** at the indicated concentrations for 24 h (**A)** or 72 h (**B**), respectively. (**C**, **D**) The normal mammary epithelial cells (WT p53 MCF10A) or normal colon cells (WT p53 CCD841), respectively, were treated with DMSO (as a control), or Ad-ISeC **14** at the indicated concentrations for 24 h (**C)** or 72 h (**D**), respectively. Percent cell proliferation determined by WST-1 was calculated as the ratio of OD_{450} values obtained for cells grown in the

presence of the Ad-ITC **6** or Ad-ISeC **14** compared with the presence of DMSO. Experiments were performed in triplicate. Error bars represents SD.

Table SI: LogP values of Ad-ITCs

Ad-ITC Number	LogP calc[a]	LogP exp[b]
1	4.53	4.46
2	4.38	4.32
3	4.54	4.5
4	4.87	4.95
5	4.82	4.76
6	5.08	5.17
7	6.21	6.34
8	4.51	4.42
9	5.33	5.40
10	6.57	6.77
11	4.65	4.51
12	3.87	3.73
13	2.92	3.11
14	3.94	4.05

[a] Calculated using Molinspiration (http://www.molinspiration.com) © Molinspiration Cheminformatics.

[b] Expeimental LogP were measured by HPLC-MS (Chiang, P.-C.; Hu, Y. Comb. Chem. High Throughput Screen. 2009, 12, 250)