

Supplementary Information

Title: An active IGF-1R-AKT signaling imparts functional heterogeneity in ovarian CSC population

Ram K Singh¹, Ajit Dhadve¹, Asmita Sakpal¹, Abhijit De² and Pritha Ray^{1*}

¹Imaging Cell Signaling and Therapeutics Lab, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Navi Mumbai, Maharashtra, India

²Molecular Functional Imaging Laboratory, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, Navi Mumbai, Maharashtra, India

* To whom request for reprints should be addressed

E-mail: pray@actrec.gov.in

Supplementary material

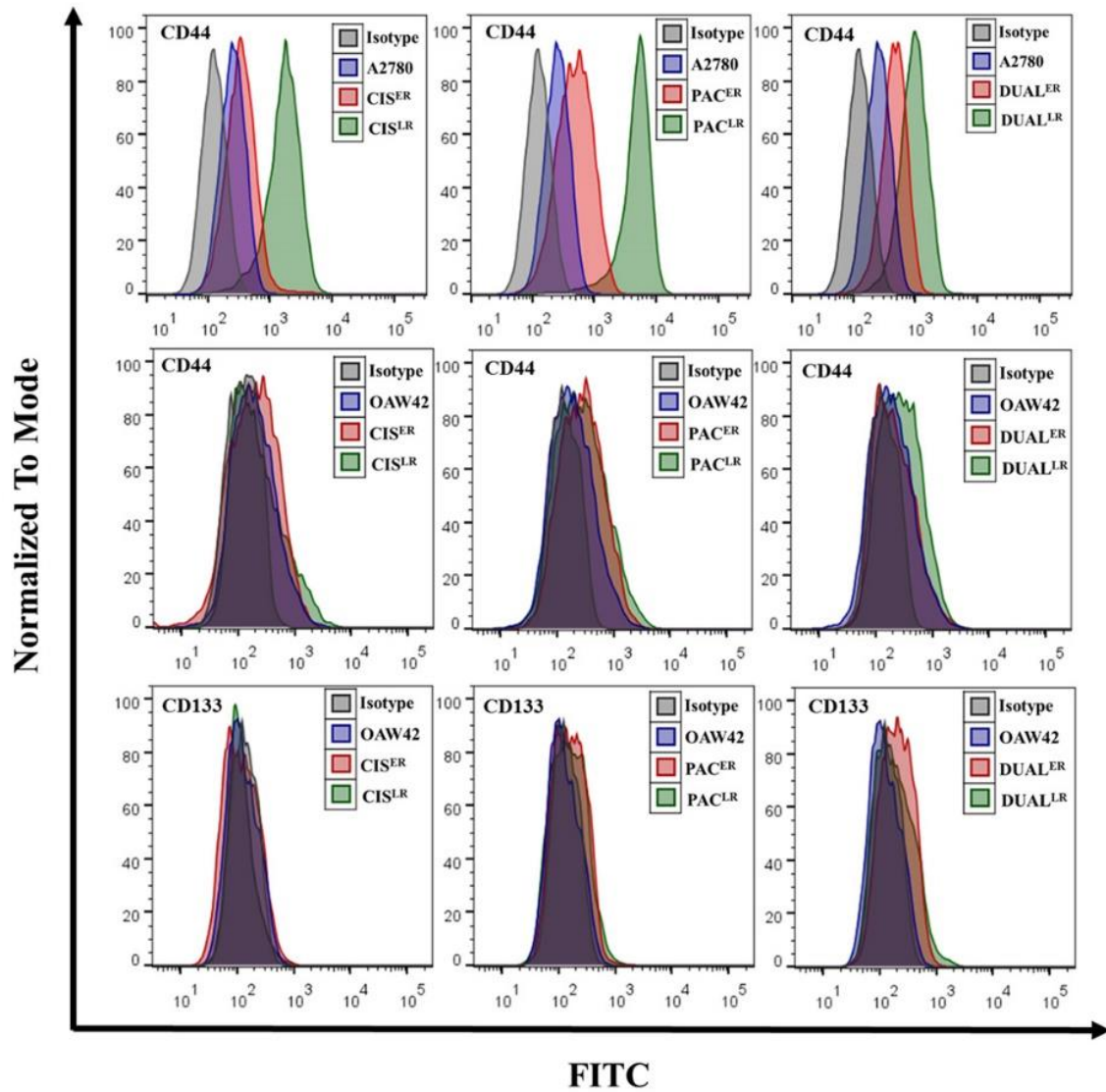
Supplementary Table 1: List of primer sequences.

IGF-1R (FORWARD)	CTGGACTCAGTACGCCGTTT
IGF-1R (REVERSE)	GGAAGTGAAGCATTGGTGCG
OCT4 (FORWARD)	GTGGAGAGCAACTCCGATG
OCT4 (REVERSE)	TGCAGAGCTTTGATGTCCTG
SOX2 (FORWARD)	AACCCCAAGATGCACAACTC
SOX2 (REVERSE)	GCTTAGCCTCGTCGATGAAC
NANOG (FORWARD)	AAAGCTTGCCTTGCTTTGAA
NANOG (REVERSE)	AAGTGGGTTGTTTGCCTTTG
GAPDH (FORWARD)	TGCACCACCAACTGCTTAGC
GAPDH (REVERSE)	GGCATGGACTGTGGTCATGAG

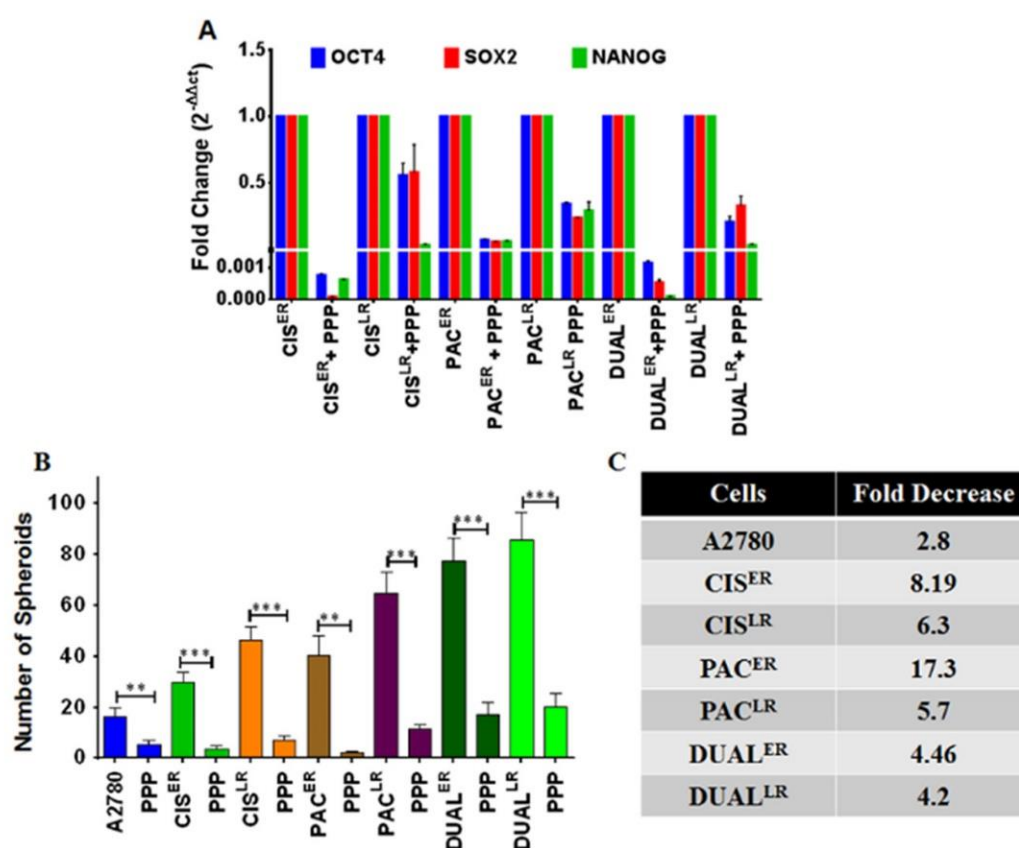
Supplementary Table 2: Percentage of SP fraction in A2780 and OAW42 resistant models

	DCV	Verapamil		DCV	Verapamil
A2780	1.5%	0.1 %	OAW 42	1.8 %	0.1 %
CIS^{ER}	3.9%	0.3 %	CIS^{ER}	2.0 %	0.5 %
CIS^{LR}	7.2%	0.1 %	CIS^{LR}	4.0 %	1.1 %
PAC^{ER}	5.5%	0.3 %	PAC^{ER}	3.2 %	0.8 %
PAC^{LR}	8.3%	0.8 %	PAC^{LR}	7.4 %	0.2 %
DUAL^{ER}	5.05%	1.9 %	DUAL^{ER}	4.6 %	0.3 %
DUAL^{LR}	19.1%	0.7 %	DUAL^{LR}	9.8 %	0.1 %

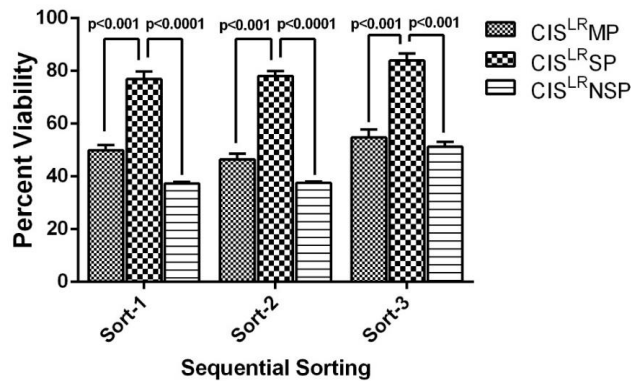
Supplementary Figure 1: Expression of CSC biomarkers: Expression analysis of CD44 and CD133 across A2780 and OAW42 resistant model showing a gradual increase with increasing resistance.



Supplementary Figure 2: Effect of IGF-1R inhibitor (PPP) on CSC phenotype in A2780 resistant models: (A) Expression analysis of pluripotent genes (*oct4*, *sox2* and *nanog*) after PPP treatment showed significant decrease in all the early and late resistant cells than their respective controls. (B) A similar trend in spheroid forming ability was graphically represented. (C) Table showing fold decrease in spheroid forming property in PPP treated cells.



Supplementary Figure 3: Cell viability assay for SP, MP and NSP fractions during subsequent sorting of SP cells. MTT assay showing significantly higher percent viability by SP cells compared to their respective MP and NSP cells during subsequent sortings (Sort1: SP Vs. NSP; $p<0.0001$, SP Vs. MP; $p<0.001$) {Sort2: SP Vs. NSP; $p<0.0001$, SP Vs. MP; $p<0.001$ } {Sort3: SP Vs. NSP; $p<0.001$, SP Vs. MP; $p<0.001$ }.



Supplementary Figure 4: Effect of IGF-1R inhibitor (PPP) in combination with AKT inhibitor in late resistant cells of A2780 resistant models:(A) MTT assay showing significant decrease in the percent viability upon combination treatment across the late resistant cells (Cis^{LR} , Pac^{LR} and $Dual^{LR}$). (B) Western Blot analysis showing decrease in the levels of both IGF-1R and pAKT upon combination treatment.

