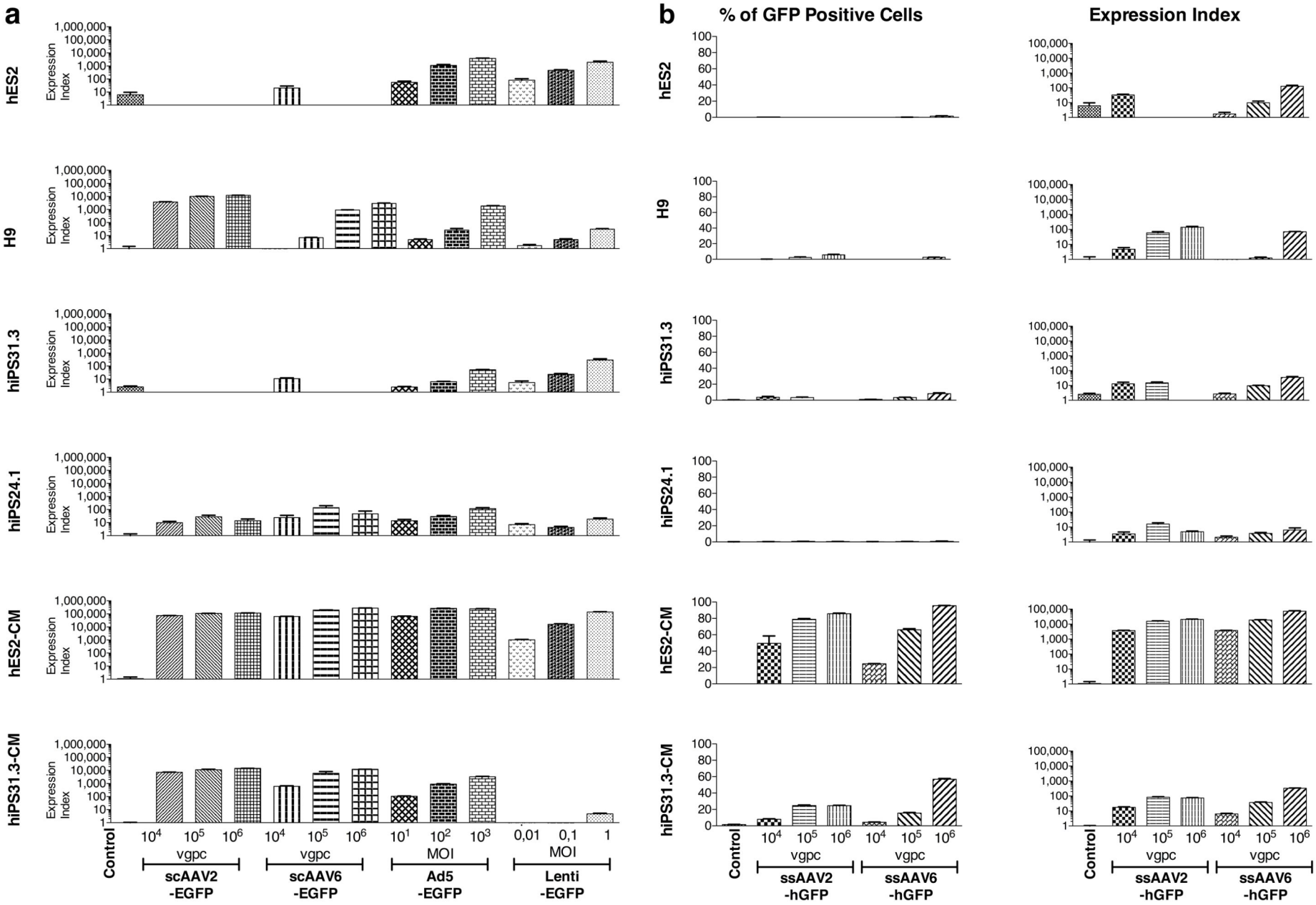
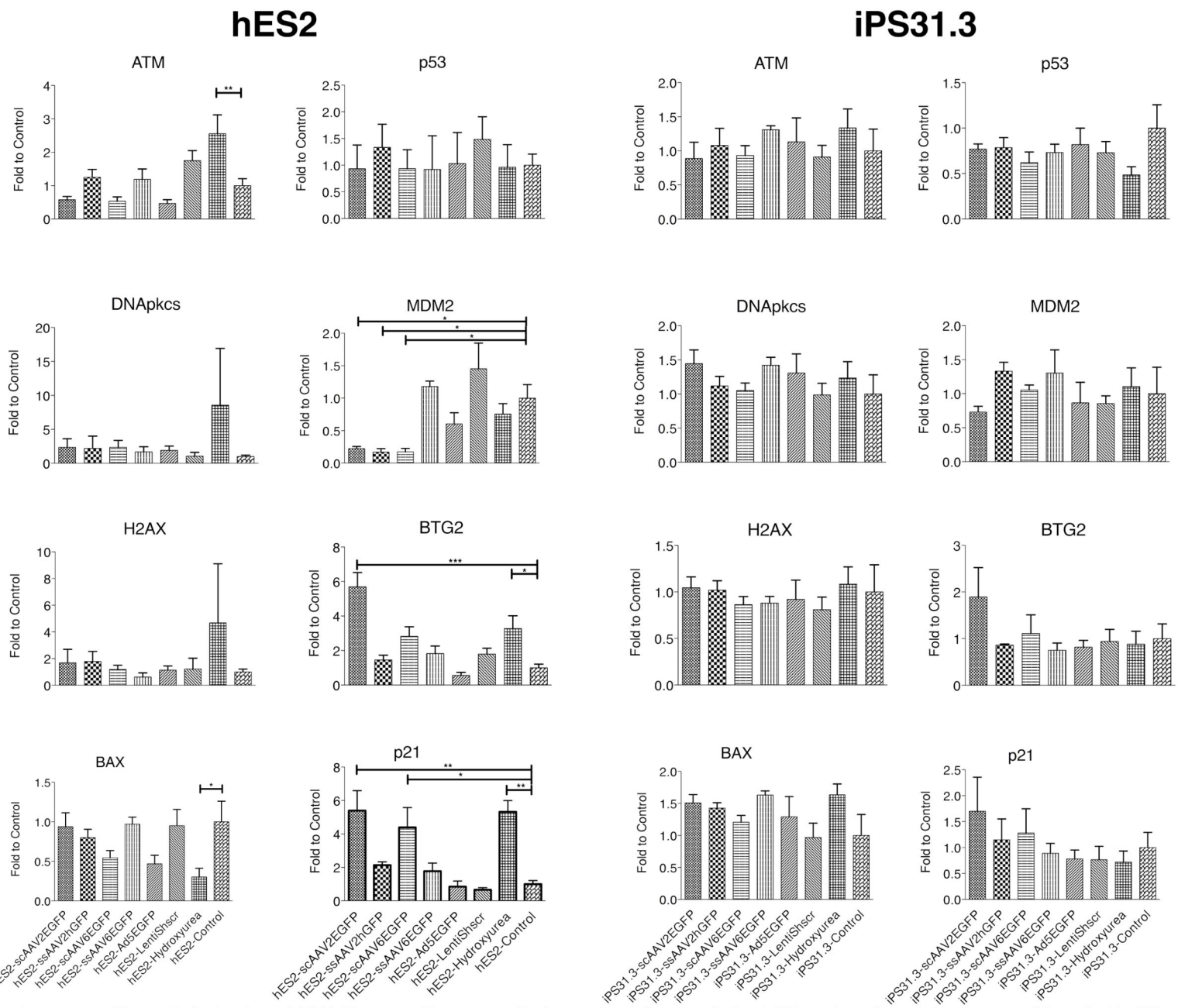


Supplementary Figure S1: Flow Cytometric Analysis of Different Pluripotent (hES2, H7, hiPS31.3, hiPS24.1) and Differentiated (hES2-CM and hiPS31.3-CM) Transduced with AAV, Adenoviral and Lentiviral Vectors.

(a)Flow cytometric analysis using the expression index (EI = (% of GFP positive cells x Mean Fluorescence Intensity (MFI))sample / (% of GFP positive cells x MFI) control), which takes into account both the number of positive cells and their mean internal fluorescence, was used to assess the transduction efficiency of different viral vectors. As shown in the graphic representation of the flow cytometric analysis, adenoviral and lentiviral vectors efficiently transduce undifferentiated cells, whereas AAVs are more efficient in transducing differentiated cardiomyocytes.
Groups iwere analyzed by ANOVA test. Please see Supplementary Table 2 for detailed statistical analysis. Bars in the graph represent standard error mean (SEM).

(b)Flow cytometric analysis of hES2, H7, hiPS31.3, hiPS24.1, hES2-CM and hiPS31.3-CM infected with single stranded AAV2 and 6 vectors carrying the hGFP transgene under the control of the CBA promoter. Self-complementary genome containing AAVs, outperform their single stranded counterparts, as shown by both % of GFP positive cells and EI. Groups were analyzed by ANOVA test. Please see Supplementary Table 1 (Luciferase, Fig1a) and Supplementary Table 2 (Flow Cytometry, Fig1b) for detailed statistical analysis.
Groups iwere analyzed by ANOVA test. Please see Supplementary Table 2 for detailed statistical analysis. Bars in the graph represent standard error mean (SEM).



Supplementary Figure 2: Induction of DNA Damage Response Pathways and Apoptosis by AAVs at low-dose infection conditions in Undifferentiated Cell Lines.) Induction of the DDR, p53 and cell cycle arrest pathways, measured by quantitative PCR (qPCR), by AAV (1 104vg per cell) but not adenoviral (MOI 1000) or lentiviral vectors (MOI 1). hES2 and iPS31.3 cells were infected with the viral vectors and 12h later the expression of several DDR, p53 and cell cycle arrest pathway genes was examined at the cDNA level. Treatment of cells with Hydroxyurea was used as a positive control. Of significance, BTG2 and p21 are upregulated significantly in hES2C cells treated for 12h with scAAV2 and scAAV6, whereas their ss counterparts only show a tendency to increase. hiPS31.3 cells were treated using the same infection conditions and were collected 24h later, however, no significant change was observed in the genes examined. Error bars depict ± SEM.

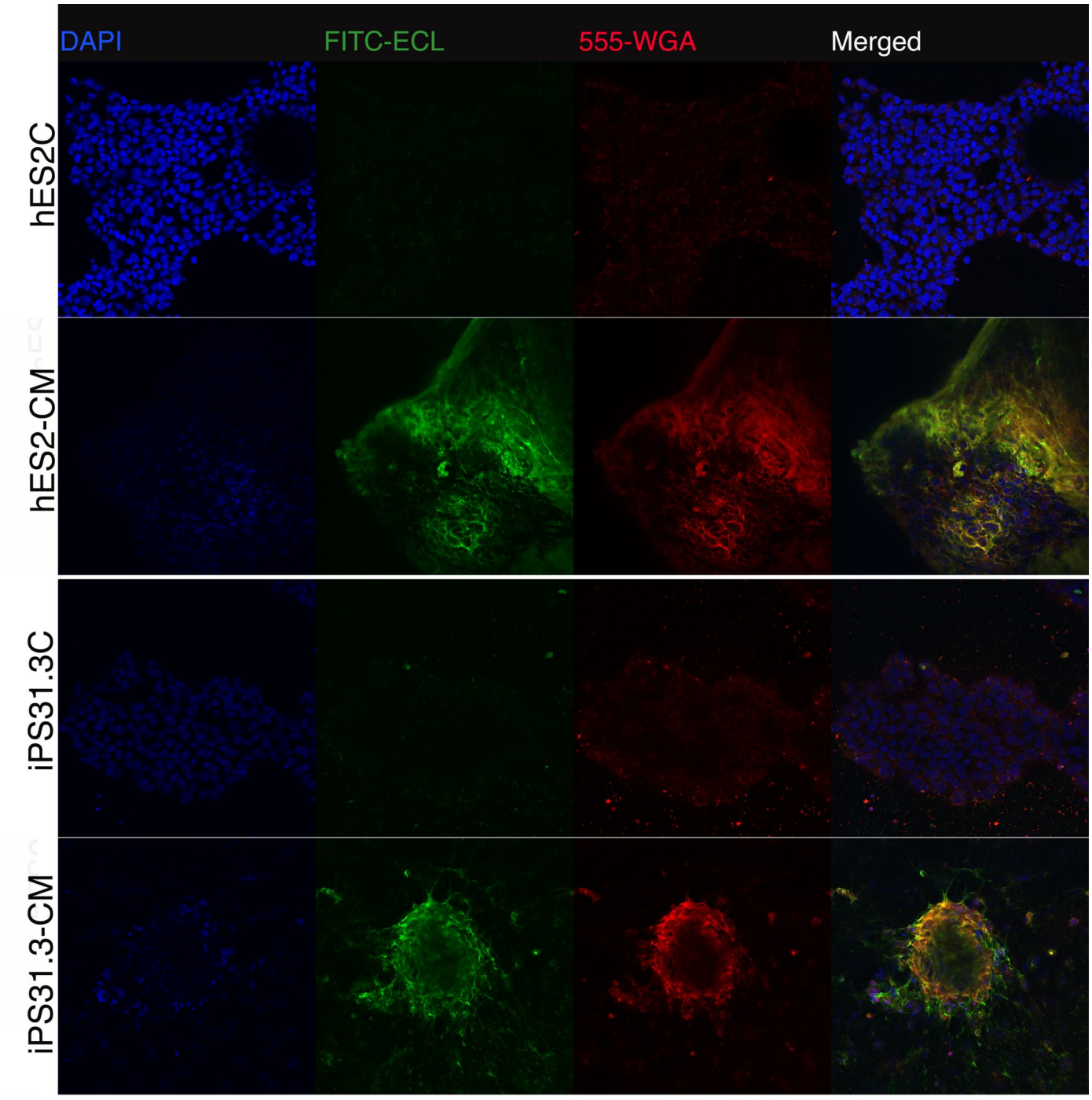


Figure S3: Identification of Receptors Against AAVs 1, 6 and 9 in Undifferentiated and Differentiated Cells. hES2c, hES2-CM, hiPS31.3c and hiPS31.3-CM were stained with Alexa Fluor® 555 conjugated WGA (Wheat Germ Agglutinin) (AlexaFluor555) and Fluorescein Labeled ECL (Erythrina cristagalli lectin). WGA staining, which targets sialic acid, the receptor for AAVs 1 and 6, was stronger in hES2 and hiPS31.3-CM, compared to PSc. ECL staining, which targets galactose, the receptor for AAV9, was undetectable at hES2c or hiPS31.3c, but strong in PSc-CM.

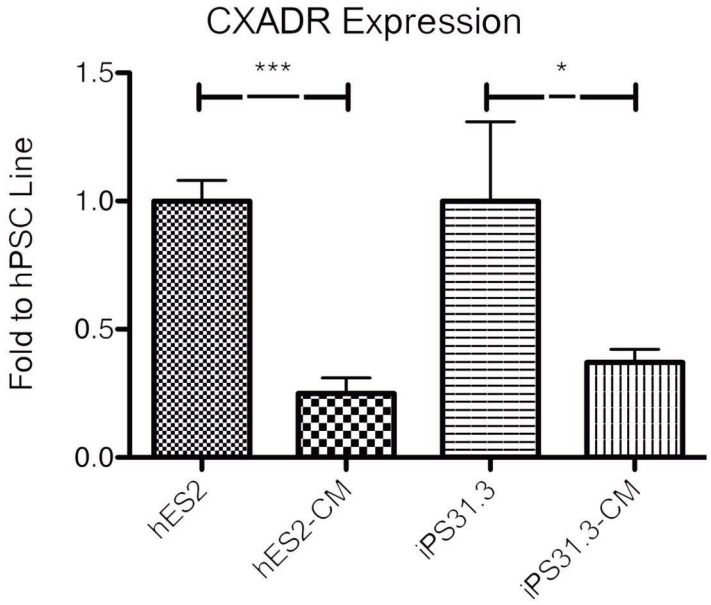


Figure S4: Expression of the Adenovirus Receptor in Undifferentiated and Differentiated Cells. Expression of CXADR in undifferentiated cell lines (hES2, hiPS31.3) and thereof derived cardiomyocytes (hES2-CM, hiPS31.3-CM) was measured by quantitative PCR. CXADR expression was detected using primers that target specifically the adenoviral-binding site on the receptor and is expressed as fold to each PSc line (hES2 and hiPS31.3). As shown, CXADR is abundantly expressed in pluripotent stem cells as well as thereof derived cardiomyocytes. Error bars depict ± SD.

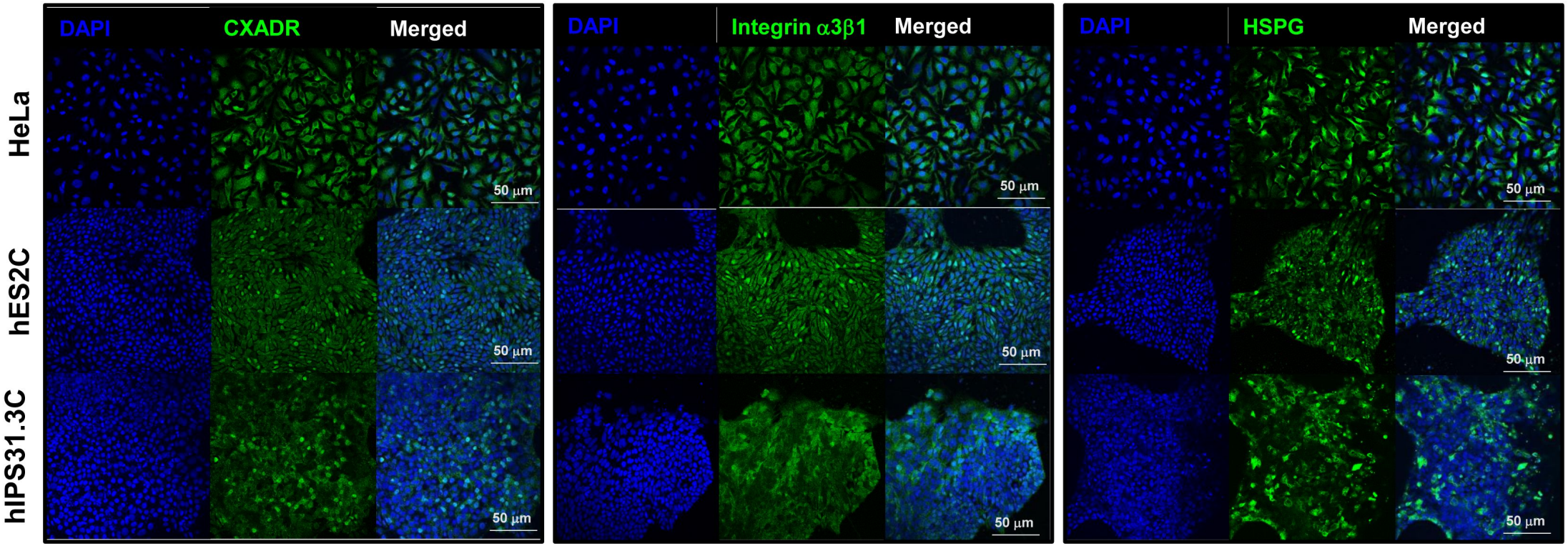
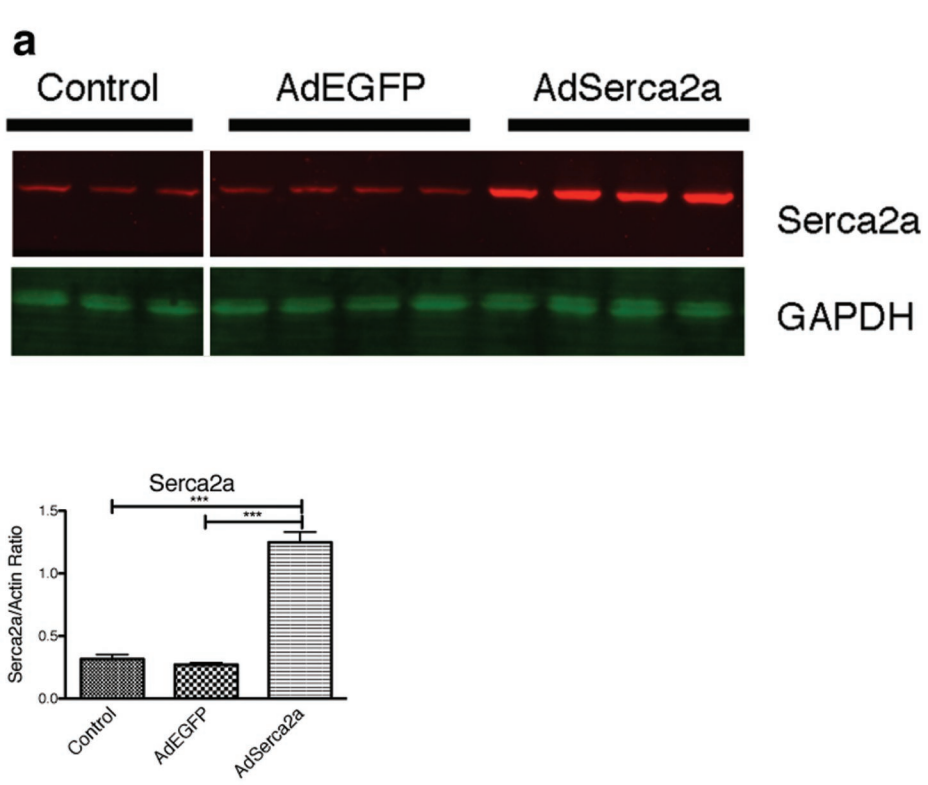


Figure S5: Identification of Receptors Against Adenovirus and AAV2 in Undifferentiated and HeLa Cells. Expression of CXADR, Integrin α1 β3 and HSPG in hPS and HeLa Cells visualzed by confocal laser scaring microscopy. HeLa cells were used as a positive control.



Supplementary Figure S6: Overexpression of Serca2a in hiPS31.3-CM moderately improves calcium cycling.
(a)Adenoviral overexpression of Serca2a in hiPS31.3-CM leads to increase in expression of a cardiac specific marker. Serca2a and EGPF, as a control, were adenoviraly overexpressed in hiPS31.3-CM. AdSerca2a infected cells showed higher expression of Serca2a.
(b)Effect of Serca2a overexpression on calcium cycling in hES2-CM. Adenoviral Serca2a overexpression in hiPS31.3-CM reduced significantly the time to maximal return velocity, but did not affect other parameters. The beating rate was reduced to the levels of hES2-CM upon Serca2a overexpression, but this change did not reach statistical significance.
Groups were analyzed by t-test. Bars in the graph represent standard error mean (SEM). Bars in the graph represent standard error mean (SEM).

Supplementary Table S3: Primers Used for Real Time PCR

Name	Forward Primer	Reverse Primer
ATM	AACGAACCTGGAGAGAGCCAAAGT	ATCAGCTGCCCTAAAGGACACAGT
PRKDC	TGGTGGCCATGGAGACTGG	GACTGGCAGAAACTGTGTAGCGGAT
H2AFX	TGT ACA CCG GCT GCT GCG GAA	TAC TCC AGC ACT GCC GCC AGG TA
TP53	AGAGCTGAATGAGGCCTTGGAACT	AATGGAAGTCCTGGGTGCTTCTGA
CDKN1A	TGGAGACTCTCAGGGTCGAAA	AGAAGATGTAGAGCGGGCCTTTGA
BTG2	TTGGAGAGAACTGTTGCGTGCTTG	TAACCTTGCTTGCTCCCTCTCACA
BAX	TCTACTTTGCCAGCAAACTGGTGC	TGTCCAGCCCATGATGGTTCTGAT
BBC3	CCCGTGAAGAGCAAATGAGCCAAA	CCCAAATGAATGCCAGTGGTCACA
NOXA1	GAGATGCCTGGGAAGAAGG	GAGTCCCCTCATGCAAGTTT
MDM2	TGCTGGGATTACAGATGTGAGGCA	ACCTTGCAACAGCTGCAGATGAAC
CXADR	TGTCGTAAAAAGCGCAGAGA	TAGGGGCAGCTACCTTAGCA