Comprehensive functional screen of extracellular vesicle scaffolds for efficient cargo delivery

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Although some tetraspanins (CD9, CD63 and CD81) have been widely used as scaffolds for extracellular vesicle (EV) detection and engineering, comprehensive functional screen of the scaffolds for efficient cargo delivery was not explored. Here, in this study, we applied the high-efficiency intracellular delivery system by engineered EVs developed in our group for comprehensive functional screen of the scaffolds. This screen pertained to all the 33 human tetraspanins, promising single-pass transmembrane proteins, cytoplasmic and membrane associated proteins. Therefore, in total 55 candidates were screened and TSPAN2 was identified as the best scaffold for intracellular delivery of cargos. We further proved the in vivo protein delivery by using intratumoral injection mouse model. TSPAN2 was also tested for Cas9/gRNA RNPs delivery in stoplight reporter cells and achieved significant effective genome editing. In addition, super-suppressor inhibitor of NF-κB was delivered by TSPAN2 engineered EVs in vitro and in vivo for decreasing NF-κB nuclear translocation and ideal treatment of LPS-induced sepsis respectively. At last, TSPAN2 engineered EVs were found to significantly increase their circulation time by displaying albumin binding domain (ABD) in the second extracellular loop to bind to mouse plasma albumin after injection. To conclude, TSPAN2 identified by comprehensive functional screen of the EV-scaffolds was a promising candidate for engineering EVs to achieve efficient cargo delivery, both intracellularly into recipient cells and on the EV-surface. TSPAN2 engineering based EVs hold potential for the treatment of a variety of protein-related diseases and the extension of EV circulation time to decrease administration frequency upon possible future clinical applications.