Background One of the major hurdles in immunotherapy is the lack of effective immunological antigens to specifically target cancer. Therefore, identification of novel tumor associated antigens (TAAs) continues to be a critically important requirement to expand the efficacy of modern immunotherapy approaches. Altered energy metabolism, excessive ROS production, and physical vicinity of the mtDNA to the OXPHOS are all factors associated with somatic mtDNA mutations in cancer. mtDNA mutation meet all the criteria for an ideal vaccine: 1. Immunogenic 2. Specific to Cancer 3. Functionally important.

Methods Our group generated a dendritic cell (DC) vaccine using lysates of mitochondria derived from RENCA cells, a spontaneously arising renal adenocarcinoma harbouring mtDNA mutations. We already published that protective immunity was observed when the vaccine was used prophylactically and therapeutically. Since we detected a higher expression of the PD1/PD-L1 pathway upon vaccination, we combined the TAMAs vaccine with an ICI treatment and we analyzed how the combination of the two treatments impact the tumor growth, the immune system, the tumor microenvironment and its vasculature.

Results The combination of the TAMAs vaccine with an ICI empowers the vaccine efficacy due to higher CD8+ infiltrate and higher expression of IFNy. Since the ability of tumor cells to capture and transfer mitochondria from neighbouring cells including endothelial cells, we analyzed a possible impact of the TAMAs vaccine re-routing toward tumor vasculature (figure 1B-C). Interestingly, in RENCA tumor bearing mice treated with TAMAs vaccine we observed significant tumor vasculature restructure and "normalization". We validate these results in vitro by mimicking the in vivo tumor to endothelial cells mitochondria transfer (figure 1A) and subsequently TAMAs specific T recognition of the endothelial cells bearing the TAMAs obtained by the mitochondrial transfer. To validate similar mechanisms in humans, we first analyzed mtDNA New Generation Sequencing (NGS) data on different cancer patients’ cohorts (total of 191) finding at least 31% of patient harbouring immunogenic mt missense mutations. Second, human healthy donor PBMCs were primed with peptides derived from these mtDNA mutations: strikingly, 100% of them responded to at least two peptides (figure 2B). Third, using patient tumors tissues we validated the mt transfer from tumor to endothelium and in line with our results, we identified specific tumor mt mutations associated with decreased tumoral CD31 staining, suggesting a specific T cell reactivity against the tumor vessels (figure 2).

Conclusions In conclusion, we validate the immunogenicity of TAMAs in human and unveil a new immunological mechanism of vasculature pruning.

Ethics Approval The study obtained ethics approval (805981) from University Laboratory Animal Resources (ULAR), that is responsible for the procurement, care, and use of all University-owned animals used for teaching, research, and testing, as approved by the Institutional Animal Care and Use Committee (IACUC) and as mandated by federal law and regulations.